OCS Study MMS 2003-071

Baseline Characterization of Anthropogenic Contaminants in Biota Associated with the Alaska OCS Liberty and Northstar Oil and Gas Production Unit in the Nearshore Beaufort Sea

Task 8 of the Arctic Nearshore Impact Monitoring in the Development Area Project (ANIMIDA)

Project Final Report

August 2003

Prepared for…
Minerals Management Service
Alaska OCS Office
Anchorage, Alaska

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This study was funded by the U.S. Department of the Interior,
Minerals Management Service (MMS), Alaska Outer Continental Shelf Region,
Anchorage, Alaska, under Contract No. 01-99-CT-30998, To 10908,
as part of the MMS Alaska Environmental Studies Program.

Baseline Characterization of Anthropogenic Contaminants in Biota Associated with the Alaska OCS Liberty Prospect and Northstar Oil Production Unit in the Nearshore Beaufort Sea

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Final Report

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Table of Contents

List of Tables

List of Figures

Executive Summary

The bioaccumulation of trace substances, including anthropogenic contaminants, was investigated in five species of fish in and near the North Slope oil field developments, including the Northstar project area and the Liberty prospect. The fish species studied were: Arctic Cod, Arctic Cisco, Four Horn Sculpin, Broad Whitefish and Humpback Whitefish. We collected fish from a total of five sites (from west to east): Stump Island, Northstar, Point Brower, Liberty and Bullen Point. Whole body concentrations of polynuclear aromatic hydrocarbons (PAH), organochlorine compounds (PCBs and 12 pesticides) and 12 trace metals were determined. Two biomarkers of contaminant exposure were also evaluated: P4501A in liver hepatocytes and gut epithelial cells and bile hydrocarbon metabolites.

The PAH, and many of the metals are potential contaminants arising from oil field operations, such as drilling, production, transportation of personnel and materials, dock construction, and onshore support operations. These contaminants also have local natural sources and distant anthropogenic sources. The organochlorines are normally not expected to result from oil field operations and most of the organochlorines undoubtedly have their ultimate sources far from the North Slope of Alaska, from where they are carried in winds and haze as volatile gases or absorbed to aerosols. However, some organochlorine data were needed for interpretation of biomarker data and their analysis did not incur much extra cost to the project. In addition, the Arctic Marine Assessment Program (AMAP), a multi-national program to assess contaminants in the Arctic, has recommended that organochlorines be measured in and around oil field operations and sites of historic military operations, e.g., Bullen Point near the development area. Further, Presidential Executive Order 12898 mandates measurement of contaminants in subsistence resources as a matter of environmental justice. In addition, distant anthropogenic sources of metals are likely making some contributions to the metals measured in biota in the development area.

The two biomarkers, P4501A induction and bile metabolites of PAH, both respond to exposure to PAH, and because of PAH metabolism, are in fact, more relevant to PAH exposure than measures of these compounds in tissues.

Site-by-site comparisons were also carried out by analysis of variance for each species to see which sites had statistically higher concentrations of contaminants. Other analyses of variance were carried out to determine if weight of fish explained a significant amount of variability in the contaminant concentration data. We report a number of different patterns of variability in which there were differences in trace substance concentrations in fish due to site and discuss site-to-site differences and whether they may be related to anthropogenic inputs. The site-to-site comparisons of trace substance concentrations in various species of fish are based on relatively small numbers of samples, so the relationships found should be interpreted cautiously, i.e. they may change with larger sample numbers. About 10% of the significant site differences in individual trace substances could be due to chance. These data and their analysis should be the basis for further investigation and monitoring. However, the data

provide a good overall picture of trace substance concentrations in fish in the development area.

Perhaps the strongest evidence for anthropogenic influences from petroleum development are the concentrations of PAH and the two biomarkers that respond to PAH, particularly in the Four Horn Sculpin. While there were no effects of site on total PAH or low-molecular weight PAH in any species of fish, there were differences seen in high-molecular-weight PAH between sites for Arctic Cisco and Four Horn Sculpin. The sites where there were elevated concentrations in these two species were Stump Island and Point Brower. In Four Horn Sculpin P4501A induction in liver varied significantly with site, with Point Brower and Stump Island having the highest responses. In Arctic Cod, Northstar fish had significantly greater P4501A content than Liberty fish. Hydrocarbon metabolites in the bile also had a pattern of differences that is somewhat consistent with P4501A induction. Phenanthrene equivalents in the bile varied significantly with site in Four Horn Sculpin, with Bullen Point, Stump Island, and Liberty having the higher responses than Point Brower. Benz(a)pyrene equivalents in the bile of this species also varied significantly with site with Stump Island clearly having the highest concentrations. Arctic Cisco also had benz(a)pyrene equivalents that varied significantly with site, with Bullen Point having the highest measured concentrations.

Both of these bile hydrocarbon components occur in extracted petroleum, and probably in drill cuttings from the developed formations, but they also have potential natural sources, particularly benzo(a)pyrene. If anthropogenic activities are responsible or contributing to these responses in Four Horn Sculpin, and given the sites at which they occur, then it is more likely that they are not directly related to drilling at the platform sites. Rather, it may be the activity to the east of Stump Island at West Dock, e.g., motorized vessels, that may be the source. There also may be some influence of the nearby Endicott Causeway on fish caught at Point Brower. At Bullen Point, historical activities at the military site may be responsible for the observed responses.

Among the chlorinated hydrocarbons and pesticides, a variety of patterns were observed. Data on site differences in these anthropogenic compounds were interpreted as to whether they may indicate distant sources, e.g., transport from lower latitudes, or some local source. In the case of PCBs, Arctic Cisco varied significantly with site; fish from Stump Island had the highest concentrations. A similar trend was seen for total pesticides, except that Stump Island and Point Brower were the two sites with the higher concentrations. However, for other individual classes of pesticides making up the total pesticide category, there were significant differences due to site with one or more species. For Chlordanes, DDTs, Endosulfans and Endrins, Stump Island had consistently high concentrations relative to the other sites. It was also mainly the Arctic Cisco that had significant site differences for these groups of compounds (save for DDTs). The Four Horn Sculpin had site differences for Chlordanes. Humpback Whitefish had site differences for Endrins. Arctic Cod had site differences for HCHs. Taken together these data suggest that there are elevated concentrations of several pesticides in the area of Stump Island and Point Brower over the general background for the area and there might be a low-level source there for pesticides. We do not know what the potential local source for this low-level elevation over background might be, but Arctic Rivers can carry significant amounts of contaminants from distant sources that are

deposited on the land and washed into the rivers during the summer. Point Brower is in the delta of the Sagavanirktok River.

Also consistent with the known long-range transport in the atmosphere from lower latitudes to the Arctic, PCBs were generally uniformly distributed among sites for four of the five species of fish analyzed, the exception being Arctic Cisco at Stump Island. We have, however, recognized two patterns of relative congener abundance in the fish from this study: a mixture dominated by high-molecular-weight congeners, and a second pattern including a similar congener composition of high-molecular-weight compounds, but also has significant, and sometimes dominant, low-molecular-weight components (e.g. IUPAC congener 8). The low-molecular-weight congeners in such mixes have been reported previously from Beaufort Sea samples.

Interpreting anthropogenic contributions of metals poses a particular problem, as the metals being analyzed all occur in the sediments and fish tissues naturally and it is possible to have residual concentrations in the gut of the fish being analyzed. We can probably discount anthropogenic loadings of nickel that did not differ with change of site. However, most of the metals did show significant differences due to site in one or two species: arsenic, barium, cadmium, chromium, iron, lead, mercury, selenium, vanadium and zinc.

The high concentrations of arsenic in Four Horn Sculpin occurred at Stump Island, Liberty and Point Brower. The reason for this pattern is not know, as it did not appear to be significantly elevated in sediments anywhere in the area on an iron-normalized basis. It is not known if there is an anthropogenic source of arsenic in the area.

Analysis of the ratios of barium to iron in the fish data suggests that sediment may have played a role in trace metals detected in Four Horn Sculpin. We therefore ascribe no other particular interpretation to the site effect seen with this element in Four Horn Sculpin. In addition, the barium sulfate used in drilling mud is not very biologically available to marine organisms.

Site effects were found for cadmium in Arctic Cisco and Humpback Whitefish. In the former species, Point Brower, Liberty and Stump Island fish had higher concentrations than Bullen Point. Stump Island Humpback Whitefish had greater concentrations of cadmium than Point Brower fish. Cadmium bioavailability can change with salinity, but it is not known if salinity was a factor in determining site differences in these two species.

There was only one species where the variation in iron content differed significantly between sites: Arctic Cod. Again, it is not known whether this is attributable to development activity at the Northstar location, but further consideration may be in order for this element as well. Since Cod are bottom feeders, at least part of the time, sediments in the gut may have influenced this outcome. Iron is an essential element and is therefore physiologically regulated in fish. It seems unlikely that without a very large biologically available source that iron would appreciably accumulate in fish beyond the range that is physiologically required.

Humpback Whitefish was the only species where site differences were seen for lead concentrations, with Stump Island fish having higher concentrations than Point Brower.

One species showed significant variation in mercury: Arctic Cisco. Point Brower Cod had greater concentrations than the other sites, but the differences in means were very little. At this stage, we cannot rule out a slight difference in natural or anthropogenic influence on mercury in this species. Nor can we rule out random variability in a small sample set (n=5-11 per site) as an explanation of this finding.

For selenium there was one species for which site had a significant effect on whole-body concentrations: Arctic Cod from Liberty had greater concentrations than those from Northstar. Again, this is based on a very small sample size (n=7-8) and we have no reason to attribute this to anthropogenic activities.

Vanadium is another element for which Arctic Cod showed significant differences due to site. In this case the Liberty fish had higher concentrations than the Northstar fish. Again the potential influence of sediments in the gut may as well as small sample size may be a factor, as there are no known sources of Vanadium at Liberty.

For zinc, the Arctic Cisco was the only species in which site had an influence on whole-body concentrations. In this case, Point Brower, Liberty and Stump Island had higher concentrations than Bullen Point. We attach no particular significance to this finding.

Based on high site-trace contaminant and site-biomarker differences, we recommend the Four Horn Sculpin as a candidate species for further monitoring of possible anthropogenic releases of trace substances that may be bioaccumulated by fish. The Four Horn Sculpin, along with the Arctic Cod, appear to be the two species that are most appropriate for monitoring at the offshore platform areas, while the two species of Whitefish and the Arctic Cisco are anadromous and more closely tied to the inshore portions of the development area. In this regard, we present and discuss the relative power of different sized collections of Four Horn Sculpin to detect changes in various trace contaminants and the number of years required to detect different percentage change with a fixed number of fish per site. For example, if Four Horn Sculpin were to be analyzed for PAH by collecting 20 fish per site per year, it is estimated that it would take 4 to 7 years of data to detect a 50% change in concentrations. The analysis of variation also indicated that 50% differences in concentrations of most analytes can be detected with fewer than 15 fish.

1.0 Introduction

Background. A new federal offshore oil production facility (Northstar) and a proposed facility (Liberty Prospect) in Alaska's North Slope prompted the Minerals Management Service (MMS) to solicit proposals to investigate the potential environmental impacts of these slated developments (since Task 8 was initiated, Northstar has been completed but Liberty is on hold). Applied Marine Sciences, Inc. (AMS), through a competitive bidding process, won the contract (Task 8) to investigate tissue contaminant residues and effects in select local biota. Task 8 addresses the critical issue of potential bioaccumulation of trace substances (both trace metals and organic compounds) in marine biota in the Liberty and Northstar development areas. This task seeks to determine whether trace substances of concern are being accumulated by higher trophic level organisms (i.e., fish and whales). Any potential effects from trace substances, whether natural or anthropogenic in origin, are not being addressed by this study. After input from the Scientific Review Board (SRB), MMS, the public and various resource agencies the study objectives now address these concerns in several species of fish. Funding and support were not provided for any further work on whales started in FY02, so this report is confined to evaluating the potential bioaccumulation of anthropogenic contaminants in several species of fish in the development area. This report describes results of analysis of fish collected in the summer of 2001.

Trace substances in the tissues of marine fish caught in the development area can come from many sources. All of the metals measured in this study have natural sources, they occur naturally in fish, and some of them are essential to fish physiology, e.g., iron, zinc and selenium. Most of the metals found in fish tissues in this study would be expected to occur in about the concentrations at which they were found. Many of these metals can also have additional sources from human activities, and this study attempts to determine if anthropogenic metal contributions were occurring by comparing tissues of fish from various areas with and without known industrial activities. In addition, it is well established that metals mobilized by industrial activities far from the North Slope are transported in the atmosphere on particles. Examples of metal sources that atmospherically transported to the Arctic include mercury from burning of coal, cadmium from smelting of zinc ores, and lead from gasoline and mining activities. These metals may enter the Arctic Ocean and be incorporated into the tissues of marine organisms.

The organochlorines are all man made. In most cases, the predominant source of organochlorines in the Arctic is long-range atmospheric transport of gases or particles from lower latitudes. Many of these compounds are volatilized at higher temperatures where they are used in large quantities (e.g. in Asia) and are deposited with snow in the Arctic. Examples of compounds found in this study that originate from distant sources include: DDT, PCBs, HCHs, toxaphene, chlordane and dieldrin. A good general reference for further information on trace substances in Arctic ecosystems is "Arctic Pollution Issues: A state of the Arctic Environment Report" (AMAP, 1997).

The polynuclear aromatic hydrocarbons in the Arctic Ocean originate from a variety of sources, including petrogenic natural sources in the area (coal and peat deposits), distant anthropogenic sources (mainly combustion products from lower latitudes), and local sources including oil and gas development activities and historic military operations.

Rationale. Due to the distribution and environmental risk of anthropogenic chemicals, these compounds (i.e., metals and organics including PAHs, PCBs and pesticides) are to be estimated in biota while oil and gas exploration and development activities on Alaska's North Slope continue. Some of these chemicals occur naturally (metals), some are imported by long-range atmospheric transport (organochlorines and some metals), others may come from historic industrial and military activity in the area (PCBs), and some may come from current industrial activity at the Northstar platform, vessel activity and other associated human activities on the North Slope of Alaska.

Objectives. The three objectives of Task 8 were to:

1. Determine the baseline concentrations of anthropogenic compounds and responses of contaminant biomarkers in representative upper trophic level biota.

2. Evaluate whether the concentrations of contaminants and/or biomarker responses indicate significant contaminant exposure to subsistence consumers.

3. Provide the essential background data and a framework for long-term monitoring of local biota if warranted by initial results of this study.

Hypotheses. In designing a study to meet these goals, AMS formulated two hypotheses:

HO_1: Baseline concentrations of PAHs, POP's, metals, and exposure/response biomarkers in biota from the Northstar and Liberty areas of the Beaufort Sea are not a result of oil and gas industry activities.

HO_2: Oil and Gas industry activities in the Northstar and Liberty areas will not result in an increase in tissue concentrations of POP's, PAHs, metals and exposure/response biomarkers in biota from the Northstar and Liberty areas.

The field collection and sampling program conducted was designed to test these hypotheses using multiple fish species at multiple sites. The six species analyzed have considerable differences in life history, site fidelity, and utilization by people.

Our choices of fish species to investigate in this study were based on several needs and goals. We set out to test our hypotheses by sampling 15-20 fish of one or two species at the Northstar Platform area and comparing them with a similar number of fish collected at the Liberty prospect area. However, as with many preliminary studies, there were significant constraints imposed on the study based on available budget, research vessels, seasonality of sampling, and the desire of MMS to provide information on fish species of importance to human subsistence fisheries. Logistical

constraints compromised this plan. Collecting time at the platform sites was limited and we did not attain our goals for numbers of fish. This was compensated for by directing more effort to other sites in the area. As a result, we have a broad sampling of species over a large area that included other reference areas, e.g., Liberty, and areas of human activity, i.e., near Stump Island and at Bullen Point. Ultimately, these collections resulted in analysis of fish that fell into two broad categories; anadromous/amphidromous and marine.

It should be emphasized that because of the potential and variable presence of distant sourced anthropogenic and naturally present compounds, in addition to those coming from petroleum exploration and production, it was not expected that all of the reference sites would have lower concentrations than the project sites for trace substances and the contaminants assayed in fish. It should also be emphasized that we are unaware at the onset of this study of PCBs and pesticides coming from petroleum production. These compounds were included in the study mainly because of their potential to affect contaminant biomarkers that would also respond to petroleum and because they are a concern for subsistence users in the area. Organochlorines have been found on oil field roads in the Kenai Peninsula where waste oil has been used for dust control. Waste fluids (not necessarily oils) were used in the Prudoe Bay field for dust control on roads (D. Prentki, MMS, personal communication). There are also confirmed reports of an organochlorine spill on the nearby Colville River (State of AK, 2000). Also, there are reports of historic spiking of the oil reservoir in the Colville river watershed with PCBs to be used as tracers. (S. Florio, personal communication).

Fish investigated in this study fall into two broad categories based on the areas of the nearshore Beaufort Sea that they inhabit. Broad and Humpback Whitefish and Arctic Cisco represent the anadromous/amphidromous fish, and the non-anadromous (marine) fish are represented by Arctic Cod and Four Horn Sculpin. The Cisco and Whitefish are the most important of these species with regard to subsistence diet and commercial fisheries, though Arctic Cod are apparently utilized for human and dog food as well. The Four Horn Sculpin has minimal importance for subsistence use (Craig, 1984, 1989). The literature on the anadromous fish is well represented by investigations of aspects of their life history, particularly with respect to effects of oil and gas industry causeways and other structures (West Dock and Endicott) on summer (open water) movements of fish (Fechhelm, 1999, Fechhelm and Bryan, 1994, Fechhelm et al, 1990, 1992, 1995, 1995, 1996, 1999, 2001, Schmidt et al, 1989, and Griffiths et al, 1992, and 1998). The two marine fish species sampled in this study (Arctic Cod and Four Horn Sculpin) are not as well represented in the literature with regard to life history or local effects, however there have been a few useful studies conducted (Barber et al., 1997; Craig, 1984; Goldberg et al., 1987; Graham and Hop, 1995).

There are several aspects of fish life history that are important when considering the measurement and interpretation of contaminants in their tissues and in attempting to determine the sources of the contaminants. These aspects are of particular importance to this study due to our investigation of both marine and anadromous fish with widely divergent life history patterns. The Cisco and Whitefish overwinter in the Sagavanirktok and, to a much larger extent, the Colville River. This means that they are only present in the project area for an average of 12 weeks per year and

are generally restricted to the narrow band of warm brackish water within a few hundred meters of the shore. The significance of this may be directly related to tissue residues of contaminants found in these fish. While it is generally understood that most tissue contaminants in fish are a result of dietary uptake, tissue residues may also be a result of contaminants passing through the gills and skin as well. Schmidt et al., (1989) described very limited feeding of overwintering Cisco and Whitefish from the Sagavanirktok River, and a slightly higher level of feeding of overwintering fish from the Colville River. Also, Kline et al. (1999) found stable isotope evidence to support some contribution of freshwater carbon to the diet of these fish species in the Prudhoe Bay area. This means that although it is likely that tissue contaminant residues in these fish are largely a result of massive summer feeding in the nearshore Beaufort Sea, some may also be reflective of winter freshwater uptake.

The regional geography and North Slope oil and gas industry developments probably also play a significant role in tissue contaminant residues with different effects on the anadromous versus marine species. Griffiths et al., (1992) claims that smaller Cisco and Whitefish collected east of West Dock are mostly fish that overwinter in the Sagavanirktok River, while those species collected west of West Dock (Stump Island) are mostly from the Coleville River. All of these issues most likely play a significant role in contaminant uptake for the fish from this study.

The Four Horn Sculpin and Arctic Cod are found further offshore and are apparently less influenced by nearshore conditions. Kline et al., (1999) characterized the carbon in Four Horn Sculpin as marine in origin and did not indicate an influence of freshwater carbon sources on this species. Though both the Arctic Cod and Four Horn Sculpin are marine fish, they appear to have widely divergent life history patterns. The Cod are more pelagic and appear to be more heavily influenced in their movements by prevailing winds than the Sculpin. They are found in large aggregations, both offshore and onshore, and during the summer may be found in large concentrations in the narrow band of brackish water along the shore. The Sculpin are found throughout the project area both onshore and offshore. In general, Sculpin species are not known for large seasonal migrations. Arctic Cod and Four Horn Sculpin have been recommended by the Arctic Monitoring and Assessment Program (AMAP) as candidate monitoring species because of their limited movement and association with the marine environment.

2.0 Methods

2.1 Fish Collections

The field program collected multiple species of fish from “impact” sites, and “reference” sites. The “impact” site for platform effects was around the Northstar production island (already under construction at the outset of Task 8). The refernce for this site was the Liberty area proposed for future development. The other sites were chosen in the course of the study to characterize the regional conditions when vessel time at the platforms restricted our collection time. These sites included Stump Island, a few miles south of Northstar and west of West Dock; Point Brower,

a few miles southeast of the Endicott Causeway in the Sagavanirktok River delta, and Bullen Point, (DEW line site) approximately 5 miles southeast of the Liberty site (Fig. 1).

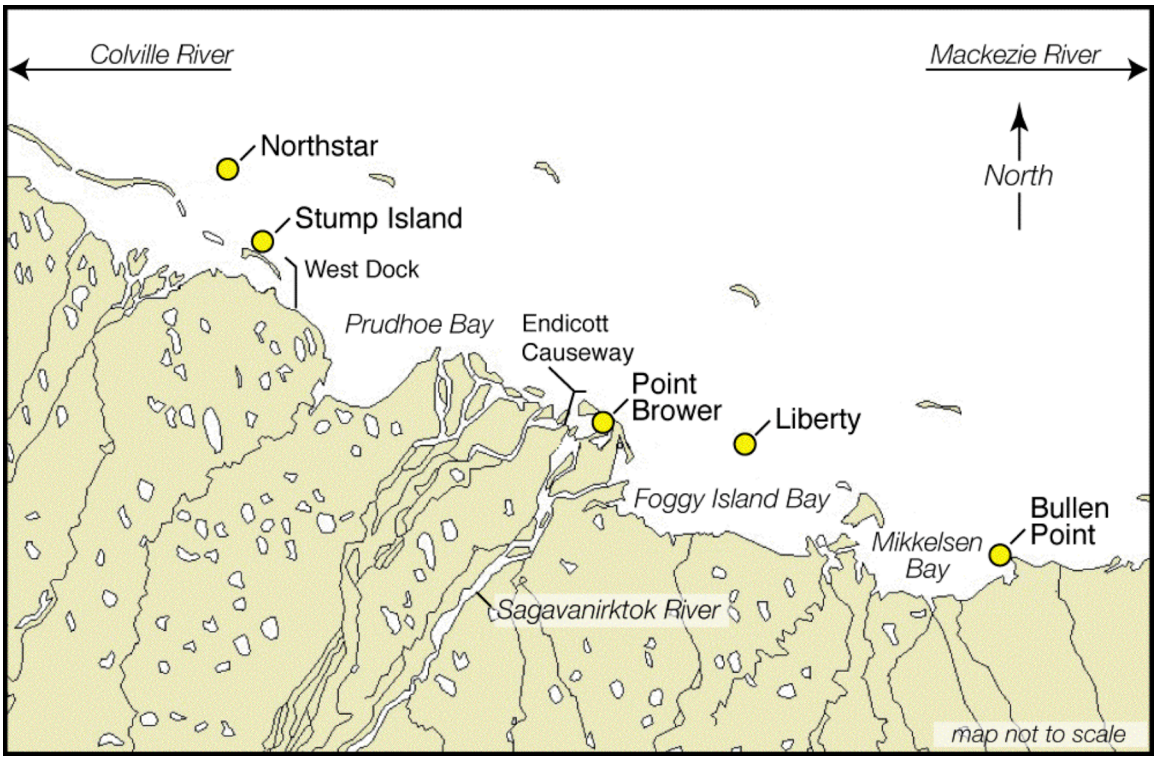

Figure 1. Locations of 2001 fish collection locations (yellow circles).

A combination of fyke net sets, gill net sets and trawl net hauls were used to capture fish in the summer of 2001 during the middle of the open water season. The field collections of fish occurred from July 25 to August 13, 2001. Methods were utilized according to site topography i.e., gill nets and trawls were used at the project sites (offshore), and fyke nets and gill nets were used at the reference sites (onshore). Fyke nets can be set in deep water by divers, however, budgetary and logistical constraints resulted in use of gill nets and trawls at Liberty and Northstar. Consideration should be given to the use of fyke nets at Northstar (and Liberty if it is completed) on the armored subsurface of the production islands for possible future collection efforts. This would require use of heavy weights to anchor the gear as rebar stakes (the traditional anchoring system for nearshore sites) could not be hammered into the concrete surface. This could be done, but would require a higher level of effort than the traditionally utilized anchoring system. Table 1 lists the latitude/longitude of each collection site and the collection method(s) employed.

Table 1. Fish collection locations and method(s)

Site Name	Location (Lat./Long.)	Method of Capture
Stump Island (SIS)	N70˚25.958′ W148˚41.922′	fyke net
Northstar (N)	N70˚29.611′ W148˚41.813′	trawl and gill net
Point Brower (PBS)	N70˚17.650′ W147˚49.211′	fyke net
Liberty (L)	N70˚16.672′ W147˚30.331′	trawl and gill net
Bullen Point (BPS)	N70˚10.524′ W146˚50.494′	fyke net and gill net

Fyke nets were deployed through use of a small outboard-engine-powered inflatable boat owned and operated by Kinnetic Laboratories Incorporated (KLI). For the Stump Island site we mobilized from West Dock. For the Point Brower site we mobilized from Endicott, and for the Bullen Point site we mobilized from the MMS launch 1273 which anchored near the site. The fyke nets were set in a similar fashion at each of the fyke net sites, though differences in site topography resulted in slight differences in the way that the nets were deployed at each site. In general, the lead was positioned perpendicular to the shoreline and started onshore, so that fish could not swim between the shore and the lead. The lead was then anchored on the bottom with re-bar stakes all the way out to the end, approximately 30 meters. The length of the lead varied at each site, dependent on water depth. The maximum water depth being approximately 120 cm, the depth that a person (of average height), wearing chest waders can safely work. Next, one side of the trap was attached to the lead, and the wing was attached to the other side of the trap. The wing was then stretched parallel to the shoreline and anchored with re-bar. The end of the wing was always positioned up current from the trap. Both the lead and the wing are rectangular nets that have corks on the top and weights on the bottom. The mesh is approximately 3 cm stretched. This method of utilizing fyke nets has been and continues to be heavily practiced on the North Slope (though often the nets are set up with two wings, so that the net will capture fish going in either direction along the shore). It is particularly effective as many of the anadromous/ amphidromous fish of the region utilize the narrow band of brackish, relatively warm water next to the shore. Fish were retrieved from the fyke nets every 24 hours, unless inclement weather precluded access to the nets. To retrieve fish from the trap, we positioned the boat next to the trap and released fish from the trap into a holding net. The fish were then immediately sorted, and any unnecessary fish were released unharmed. The fish that were kept for the study were placed in a clean seawater filled plastic tub with a lid, and transported as quickly as possible to the dissection lab.

Trawls were conducted around both the Northstar production island and the area slated for the Liberty project. The net was a small (approx. 3 m width) scientific sampling otter trawl set up for bottom trawling (as opposed to mid-water trawling). The net was deployed from the MMS launch 1273, an 11m-long aluminum vessel with a draft of 1.3 m. The net was deployed and retrieved with a single winch wire that was tied to the trawl doors. Tows were typically 1 mile in length and speed over bottom averaged approximately 2.5 knots. Water depth varied from approximately 5-15 m. Contact with the bottom was determined by inspecting the polishing of the metal runners on the bottom of the trawl doors. Additional information about the location, duration, speed and depth of the individual tows may be found in Table 1

and in Appendix A. Note that Table 1 only lists one trawl location for each trawl site. There were actually multiple trawl locations for each trawl site but they were all very close together.

Gill nets were deployed at Northstar, Liberty and Bullen Point. The nets were cut from old commercial herring nets formerly used in the San Francisco Bay (CA) fishery. They are knotted monofilament nets of approximately 5.5cm (stretched) mesh, an appropriate size for collection of most of the Beaufort Sea fishes, with the exception of young of the year Coreogonids and Char, and small Cod and Snailfish. The nets were approximately 70m long and 8m deep and were rigged with anchors and floats, enabling them to sit on the sea floor. They often fished the entire water column as they were typically set in less than 8m of water, and the fairly light currents did not lay them over.

Catch per unit effort (CPUE) was determined for each of the methods. These determinations do not include mobilization and demobilization times (which differ widely for each method), and include the entire catch for each method, rather than just the fish that were selected for the study. Additionally, the CPUE's were determined for each gear type, and included all use of each gear type at all sites. The CPUE for fyke nets was 1.5 fish per hour, for gill nets was 10.6 fish per hour, and for trawls was 17.7 fish per hour.

Due to the uncertainty in knowing which species and how many fish would be captured at each site, a flexible approach was taken for species selection. In addition to the objectives previously identified, goals for fish collections included:

1. To sample fish species that are important components of local human subsistence diets, and
2. To sample species with maximum local site fidelity, so that their tissues might reflect uptake of local sources of contaminants.

These additional goals were met despite the relatively depauperate nature and low diversity of local fish populations. We kept most of the fish captured at each site, then chose the most appropriate species for analysis after the collections were completed. Multiple fish species were captured using fyke nets, otter trawls, and gillnets. Of these, eight species were collected and dissected: Four Horn Sculpin (*Myxocephalus quadricornus*), Arctic Cod (*Boreogadus saida*), Broad Whitefish (*Coreogonus nasus*), Humpback Whitefish (*Coreogonus pidschian),* Arctic Cisco (*Coreogonus autumnalis*), Dolly Varden Char (*Salvelinus malma*), Arctic Flounder (*Pleuronectes glacialis*), and Snailfish (*Liparidae*). The Dolly Varden, Arctic Flounder, and Snailfish were not analyzed. Their tissues have been archived for potential future analysis. The original design of the field program was based on obtaining two years of data in 2000 and 2001, so that comparisons between years could be made. It was thought that potential differences in tissue contaminant residues and biomarker data from species collected at the same locations during ensuing field seasons might allow some determination of annual variability in contaminants. The original design was changed due to poor fish catches during the first field season in 2000. The poor catch was a result of several factors, including an enforced late start to the field season and limited access to the vessel in order to accommodate the needs of the other tasks. These issues were overcome to some extent during the second field season, during

which more successful fish collections were made at multiple locations. The reported data are therefore from 2001 samples only.

2.1.1 Fish Dissections and Sample Storage

Dissections began as soon as possible after the daily fish collection was completed and the fish were brought to the dissection area. Fish collected at Stump Island and Northstar were dissected at British Petroleum's (formerly ARCO) seawater treatment plant at the end of West Dock. Fish collected at Point Brower and Liberty were dissected at Endicott, and fish collected at Bullen Point were dissected on the MMS launch 1273.

Jordan Gold, one of the Task 8 investigators, directed the dissections and was assisted by either two or three other ANIMIDA scientists depending on availability of personnel. One individual did all of the record keeping, filled out the dissection forms and made the labels for all of the tissue containers. Another individual removed fish from the holding tank, sacrificed them with a sharp blow to the head, and then weighed and measured the fish before providing them to the dissector. When only three people were involved, the same individual then positioned the containers for the deposit of the tissues provided by the dissector, and sealed each container after it was filled with the appropriate tissue and fixative (if used). The same individual also removed bile from the gall bladder with the help of the dissector, and deposited the bile into the bile container (a factory cleaned small glass vial with a Teflon lined cap) and then placed the container on dry ice. When a third scientist was available, that person took over the duties of processing the bile samples.

Dissections were accomplished in a very similar fashion regardless of the species being dissected. This description is for the basic process, without regard to minor differences required by the different species. Prior to the beginning of the dissections, a brief meeting was conducted to assign duties and discuss any issues for the upcoming dissections, including the order in which each species would be dissected. This was of issue as free flowing seawater was not available, so as the dissection process wore on, the water quality in the fish holding tank degraded. To minimize the chances of the fish dying prior to the end of the dissections, assessments were made as to the relative "toughness" of the individual fish species. The order in which fish were removed from the tank was generally as follows: Dolly Varden (Char), Arctic Cisco, Arctic Cod, Humpback Whitefish, Broad Whitefish, Snailfish, Arctic Flounder, and Four Horn Sculpin. This order varied on a daily basis, as all species were not caught during each collection event. Once the order of dissections was determined and everyone was ready, the fish dissections began. In general, each dissection took approximately 3 minutes, with approximately 3 minutes in between each dissection to store the dissected tissues, rinse the dissection gear and get the containers labeled for the next dissection. Once the dissections began, they continued non-stop until all fish were processed and tissues were stored.

The required containers were labeled, all dissection gear was detergent washed, rinsed with deionized (DI) water, washed with dilute HCL, rinsed with DI water,

washed with methanol and rinsed with DI water. All of the personnel handling tissues wore nitrile gloves and washed and rinsed their gloves in the same fashion as the dissection instruments. This process for cleaning gear/gloves was repeated prior to handling each fish. After being removed from the holding tank, sacrificed, measured on a millimeter scale plastic measuring board, weighed on a pesola balance (100g or 20kg scale depending on fish size), the fish was placed on the nylon dissection board. The dissector then snipped off a small piece of gill arch for P4501A, and opened up the fish by slicing from the anus through the pectoral girdle and up to the gills with a scalpel, thus exposing the organs within the peritoneal cavity. Depending on species and fish size, a second cut was sometimes made through the musculature dorsal of the pectoral girdle to facilitate exposure of the peritoneal cavity. The gall bladder was then located and bile was removed through the use of a small gauge pre-cleaned syringe. A snip of liver, gut, heart (whole heart in small fish), kidney, spleen, muscle (from the belly) and gonad was then removed for P4501A, and all P4501A samples were placed in an HDPE container. The sex was visually determined if possible, notes were made about irregularities (such as fin deformities, lesions, abnormal characteristics, or swollen or discolored organs), peritoneal fat and parasites, and the remaining carcass was then either placed in a factory-cleaned glass container, or if too large, was wrapped in combusted foil and then placed in a Ziploc bag for future organics and metals analyses. The carcasses were placed on dry ice if on the boat, or in a freezer if ashore. The bile was frozen on dry ice prior to being placed in a freezer. The P4501A samples were fixed in 10% formalin in seawater. Additionally, rinse blanks (equipment blanks) of the dissection gear were periodically taken by collecting DI water poured over cleaned dissection gear to ensure that the cleaning procedures were adequate, and that they were not contributing (assayed) contaminants to the tissue samples. These blanks were collected into factory cleaned glass containers, and were frozen for shipment to the analytical labs.

At the end of the field season, P4501A samples and frozen bile samples (packed with dry ice) were shipped to AMS's facilities in California and the frozen carcasses were shipped to ICF's facilities in Massachusetts. On arrival at the laboratories, the samples were logged in and either placed in freezers (carcasses and bile) or stored at room temperature (P4501A).

The determination was then made as to which samples should be analyzed. ICF was notified and they homogenized the carcasses and archived portions in their freezers for possible metals analyses. These archived portions were later shipped to Dr. John Trefry's lab in Melbourne, Florida (Florida Institute of Technology) where the metals analyses were conducted. AMS shipped the bile samples to Dr. Peggy Krahn's lab in Seattle, Washington (National Marine Fisheries Service), and the P4501A samples to Dr. John Stegeman's lab in Woods Hole, Massachusetts (Woods Hole Oceanographic Institute).

Though notes were taken of parasites, deformities/irregularities, and peritoneal fat, no analysis was undertaken that utilized this information. These notes are in the individual dissection sheets in the appendices.

2.2 Analyses of Contaminants and Biomarkers

The analyses included organic (i.e., petroleum and non-petroleum related) and metal parameters in tissues, bile FAC's (a marker of short-term on time scales of hours to days, exposure to aromatic hydrocarbons), and the biomarker CYP1A (a marker of exposure to aromatic and some chlorinated hydrocarbons, e.g., coplanar PCBs, for periods of days to weeks). Analytes are listed in Table 2.

Table 2. List of all assayed analytes.

Whole Body Organic Compounds

8 - 2,4'-Dichlorobiphenyl (Cl2)	lindane
18 - 2,2',5-Trichlorobiphenyl (Cl3)	Mirex
28 - 2,4,4'-Trichlorobiphenyl (Cl3)	Toxaphene
44 - 2,2',3,5'-Tetrachlorobiphenyl (Cl4)	Naphthalene
52 - 2,2',5,5'-Tetrachlorobiphenyl (Cl4)	Benzo[g,h,i]perylene
66 - 2,3',4,4'-Tetrachlorobiphenyl (Cl4)	Biphenyl
101 - 2,2',4,5,5'-Pentachlorobiphenyl (Cl5)	C1-Naphthalenes
105 - 2,3,3',4,4'-Pentachlorobiphenyl (Cl5)	C2-Naphthalenes
118 - 2,3',4,4',5-Pentachlorobiphenyl (Cl5)	C3-Naphthalenes
128 - 2,2',3,3',4,4'-Hexachlorobiphenyl (Cl6)	C4-Naphthalenes
138 - 2,2',3,4,4',5'-Hexachlorobiphenyl (Cl6)	Acenaphthylene
153 - 2,2',4,4',5,5'-Hexachlorobiphenyl (Cl6)	Acenaphthene
170 - 2,2',3,3',4,4',5-Heptachlorobiphenyl (Cl7)	Anthracene
180 - 2,2',3,4,4',5,5'-Heptachlorobiphenyl (Cl7)	Dibenzothiophene
187 - 2,2',3,4',5,5',6-Heptachlorobiphenyl (Cl7)	C1-Dibenzothiophenes
195 - 2,2',3,3',4,4',5,6-Octachlorobiphenyl (Cl8)	C2-Dibenzothiophenes
206 - 2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (Cl9)	C3-Dibenzothiophenes
209 - 2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (Cl10)	Fluorene
2,4'-DDD	C1-Fluorenes
2,4'-DDE	C2-Fluorenes
2,4'-DDT	C3-Fluorenes
4,4'-DDD	Phenanthrene
4,4'-DDE	C1-Phenanthrenes/anthracenes
4,4'-DDT	C2-Phenanthrenes/anthracenes
Aldrin	C3-Phenanthrenes/anthracenes
alpha-Chlordane	C4-Phenanthrenes/anthracenes
cis-Nonachlor	Benzo[a]anthracene
gamma-Chlordane	Chrysene
Heptachlor	C1-Chrysenes
Heptachlor Epoxide	C2-Chrysenes
Oxychlordane	C3-Chrysenes
Methoxychlor	C4-Chrysenes
trans-Nonachlor	Fluoranthene

alpha-hexachlorocyclohexane	Pyrene
beta-hexachlorocyclohexane	C1-Fluoranthenes/pyrenes
delta-hexachlorocyclohexane	C2-Fluoranthenes/pyrenes
Endosulfan I	C3-Fluoranthenes/pyrenes
Endosulfan II	Benzo[a]pyrene
Endosulfan Sulfate	Benzo[e]pyrene
Dieldrin	Benzo[b]fluoranthene
Endrin	Benzo[k]fluoranthene
Endrin Aldehyde	Dibenzo[a,h]anthracene
Endrin Ketone	Perylene
hexachlorobenzene	Indeno[1,2,3,-c,d]pyrene

Bile Fluorescent Compounds

PHN Equivalents (ng/g)	BaP Equivalents (ng/g)

P4501A Response

liver hepatocytes	skin/muscle vascular endothelium
liver vascular endothelium	gonad vascular endothelium
liver bile ducts	gut mucus epithelium
gill pillar cells	gut vascular endothelium
gill epithelium	spleen vascular endothelium
gill vascular endothelium	heart atrial endothelium
kidney tubules	heart ventricle endothelium
kidney vascular endothelium	

Whole Body Metal

arsenic	mercury
barium	nickel
cadmium	lead
chromium	selenium
copper	vanadium
iron	zinc

2.2.1 Tissue Organic Residue Analyses

Tissue organic residue analysis was conducted through analysis of PAHs with GC/MS (gas chromatograph mass spectrometry) and the PCBs and pesticides through GC/ECD (gas chromatograph electron capture detection). The PCB and pesticide data reflect individual lifetime exposure to non-metabolized organic compounds, while PAH residues reflect some fraction of accumulated PAH, i.e., the non-metabolized fraction.

This section describes the sample preparation and analytical methods that were used in performing the organic chemical analyses.

2.2.1.1 Tissue Sample Preparation

The entire fish body was homogenized for all samples. A 5-gram aliquot was removed from each homogenized fish sample, frozen, and sent to John Trefry at Florida Institute of Technology (FIT) for metals analysis. Since metals determinations were to be performed on the homogenized tissue samples, the use of stainless steel utensils were avoided when handling the tissue samples. Whenever possible, Teflon® coated or titanium utensils were used to handle the tissue samples.

Samples were grouped together into four batches of 20 or fewer samples plus associated quality control samples. The level of contamination in the samples was expected to be low. A procedural blank (PB), blank spike (BS), laboratory duplicate, and tissue standard reference material (SRM) sample were extracted with each batch of tissue samples.

A 5-gram aliquot of each homogenized sample was removed for dry weight determination. Approximately 15 grams wet weight of tissue homogenate was transferred to a clean Teflon® centrifuge tube for digestion. The remainder of the homogenate was re-labeled and stored frozen as archived samples.

Thirty (30) mL of pre-extracted 6N potassium hydroxide and the surrogates were added to each homogenized tissue sample. The surrogates used were naphthalene-d8, acenaphthene-d10, phenanthrene-d10, and benzo[a]pyrene-d12 for PAH analysis and dibromo-octafluorobiphenyl, PCB 103, and PCB 198 for PCB and pesticide analysis. Surrogate compounds were spiked into all tissue samples at the low-level because target compound concentrations in the samples were expected to be at trace levels. The container was then flushed with purified nitrogen, sealed, and allowed to digest overnight in a hot water bath at approximately 35°C. After digestion, 30 mL of ethyl ether was added to each sample and the mixture was agitated on an orbital shaker for 5 minutes. The samples were then centrifuged at 2,000 revolutions per minute (rpm) for 5 minutes to facilitate phase separation. The ether layer was removed using a Pasteur pipette and filtered through sodium sulfate into a 250-mL K-D apparatus. The ether extraction of the digest was repeated twice, and the ether extracts combined in the K-D apparatus. The combined ether extract from each sample was reduced in volume to approximately 1 mL by K-D and nitrogen concentration techniques. The extracts were then exchanged to methylene chloride

and an aliquot was removed and weighed on an electrobalance for total non-saponifiable, lipid-weight determinations.

2.2.1.2 Extract Fractionation

The fish tissue extracts were fractionated in order to remove potential interference and to improve the quality of the analysis at trace levels. Prior to fractionation, the sample extracts were exchanged from hexane to methylene chloride under nitrogen.

The fractionation was performed using a 30-cm by 1-cm column that was wet-packed in methylene chloride with 100 percent activated silica gel/5 percent deactivated alumina/activated copper (approximately 11:1:2 g) and preconditioned with 30 mL methylene chloride followed by 30 mL of hexane. The sample extract (which had been verified to be less than 50 mg extractable material per 1 mL) was loaded onto the column. The sample was eluted with 18 mL of hexane and the isolated saturate (f1) fraction was collected. This was followed by 21 mL of hexane:methylene chloride (1:1) to isolate the aromatic (f2) fractions.

2.2.1.3 Internal Standard Addition

The extracts (or extract fractions) were reduced to a measured final volume under a stream of nitrogen. The final sample extracts were spiked with PAH and pesticide/PCB internal standards, as appropriate for each extract or fraction. In general, the extracts were concentrated to approximately 250 microliter (μL) before adding the internal standards in order to lower detection limits. The internal standard compounds used were chrysene-d12 and fluorene-d10 for PAH analysis and tetrachlorometaxylene for pesticide/PCB analysis.

2.2.1.4 Organic Instrumental Analysis

Instrumental analysis of the tissue samples was performed by GC/MS for PAHs and by GC/ECD for pesticides and PCBs. The laboratory SOPs include the acceptability criteria for the calibration, procedural blank, surrogate compound recoveries, and spike recoveries, as well as the corrective action if the criteria are not met, reporting requirements, and method detection limit (MDL) protocols. The data quality objectives (DQO) for these analyses are summarized in Tables 3 and 4.

2.2.1.5 Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry

Analysis for PAHs was performed according to ICFs SOP ICF-2827, "Determination of Polynuclear Aromatic Hydrocarbons and Selected Heterocyclic Compounds by Gas Chromatography/Mass Spectrometry in the Selected Ion Monitoring Mode." (see US EPA 1993; method 8270). ICF's PAH analysis method is a modified version of EPA's SW-846 Method 8270. The target PAHs compounds are listed in Table 2. The GC/MS was operated in selected ion monitoring (SIM) mode to obtain the desired sensitivity. The GC/MS was first tuned with perfluorotributylamine (PFTBA) to verify accurate mass assignment and to maximize the sensitivity of the instrument in the mass range of interest (100 to 300 atomic mass units). After tuning, an initial calibration was performed which consisted of five calibration standards, at different

concentration levels, spanning the concentration range of interest. Average response factors for each target compound and surrogate are calculated from the initial calibration standards relative to the internal standard compounds added to the sample extracts just prior to instrumental analysis (internal standardization). Continuing calibration standards, at a mid-range concentration level, were analyzed every 18 hours or after every 12 sample analyses to monitor sensitivity and linearity of the GC/MS. The average response factors generated from the initial calibration were used to calculate the concentrations of target compounds and surrogates in the environmental and quality control samples. The recoveries of the surrogate compounds spiked into the samples prior to extraction were used to assess sample-specific extraction efficiency. The target compound concentrations were adjusted based on sample-specific surrogate recoveries to correct for differences in extraction efficiency (surrogate corrected).

2.2.1.6 Chlorinated Pesticides and PCB Congeners by Gas Chromatography/Electron Capture Detector

Analysis for pesticides and PCBs was performed according to ICFs SOP ADL-2818, "Determination of Chlorinated Pesticides and PCB Congeners by Gas Chromatography/Electron Capture Detection." (see US EPA 1993; method 8081/8082). ICF's pesticide and PCB congener analysis method is a modified version of EPA's SW-846 Method 8081 using dual, dissimilar columns and dual detectors. A Restek RTX-5 column (or equivalent) was used as the primary column and a DB-17 column (or equivalent) was used as the confirmation column. The target pesticide and PCB congeners compound lists are listed in Table 2. Prior to sample analysis, an initial calibration was performed which consisted of five calibration standards at different concentration levels ranging from 1 to 200 ng/mL. Average calibration factors for each target compound and surrogate were calculated from the initial calibration standards (external standardization). Continuing calibration standards, at a mid-range concentration level, were analyzed every 18 hours or after every 10 sample analyses to monitor sensitivity, retention time stability, and linearity of the GC/ECD. Sample analyses were performed after acceptable calibration analyses were obtained. The average calibration factors generated from the initial calibration were used to calculate the concentrations of target compounds and surrogates in the environmental and quality control samples. When coelution occurred between one or more target compounds or when interference occurred on the primary column, the results were reported from the confirmation column for the affected compounds. Compound identification was based on 1) detecting a peak within the established retention time window for a specific compound on both the primary and confirmation columns and 2) the analyst's judgment. The recoveries of the surrogate compounds spiked into the sample prior to extraction were used to assess sample-specific extraction efficiency. The target compound concentrations were adjusted based on sample-specific surrogate recoveries to correct for differences in extraction efficiency.

2.2.1.7 Organic Chemistry Laboratory Quality Control

Data Quality Objectives and Quality Control Samples
A set of DQOs was established for the program to ensure that the analytical data would be of the quality necessary to achieve the project objectives. The DQOs were adapted from the specific laboratory analytical SOPs and were included in the laboratory workplan specific for the program. They are included here as Tables 3 and 4. Tables 5 and 6 summarize the quality control results for PAH's, PCB's and pesticides.

For processing, samples were grouped together in batches of 20 field samples, plus associated QC samples. In general, the QC samples processed with each batch of tissue samples included one procedural blank, one blank spike, one SRM (Tissue SRM 1974a), and one duplicate analysis. The blank spike sample was fortified with PAH, pesticide, and PCB matrix spike solutions.

There were a number of additional measures added to the processing of the samples to monitor QC and to aid in the assessment of the data's usability with respect to the program objectives. An important part of this is the evaluation of specific QC samples for accuracy, precision, and potential contamination. The following is a general description of some elements.

2.2.1.8 Solvent and Standard Checks

Prior to sample analysis, every lot of solvent used in the analytical process was analyzed in duplicate to verify that it was free of contamination and acceptable for use. Likewise, prior to spiking the samples with surrogates and internal standards, all standard preparation records were checked. No standards were used for an analysis unless they had been approved for use.

2.2.1.9 Instrument Calibration

Before instrumental analysis of sample extracts, a multi-level calibration was analyzed and the linearity of the analyte response factors were evaluated. A continuing calibration standard was analyzed regularly to check the stability of the instrument response. If the relative standard deviations (RSDs) for the initial calibration or the percent difference of the daily calibration did not meet the criteria set in the SOP, a new calibration was run and the affected samples re-analyzed.

2.2.1.10 Reference Samples (for PAHs)

To assess the accuracy of the mixture used to calibrate the method, an independently verified instrument standard reference material (IRM) was analyzed against the calibration standard for PAH samples. The values of the analytes had to be within 15 percent of the target value for the calibration solution to be valid.

In addition, a solution of an assayed crude oil was analyzed with each initial calibration sequence and the results were compared to a laboratory-established mean

to assess method accuracy. The solution was also used to provide petroleum pattern information and to aid in qualitative identification of target compounds.

2.2.1.11 Procedural Blank

A procedural blank was processed and analyzed with each analytical batch in order to monitor potential contamination resulting from laboratory solvents, reagents, glassware, and processing procedures.

2.2.1.12 Blank Spike

A blank matrix was spiked with representative target compounds prior to extraction to assess the effect of the sample processing procedure independent of sample matrix effects.

2.2.1.13 Laboratory Duplicate

A field sample was analyzed in duplicate to assess the precision of the method in the target matrix.

2.2.1.14 Standard Reference Materials

A Standard Reference Material of a well-characterized sample of known concentration was processed through sample preparation and instrumental analysis with each batch of samples. The results were compared to externally certified values to assess method accuracy. This program used tissue SRM 1974a provided by National Institute of Standards and Technology (NIST).

2.2.1.15 Laboratory Records

The laboratory maintained detailed records throughout the processing of the samples. All raw instrumental data were archived electronically. Completed records or copies of forms were collated into a binder for final archive storage. The final laboratory data package contains sufficient detail so that an external audit could be performed. The documentation in the final data package includes:

- Lot numbers, vendor, and preparation records for reagents and standards
- Sample preparation records
- Analytical procedures used that are not documented in laboratory SOPs
- Instrument analysis records
- Instrument raw data hardcopy
- Documentation of observations or deviations encountered

2.2.1.16 Laboratory Data Review

The following describes the process of data reporting and review by the laboratory. The chemistry data for each analysis were reduced and reviewed by the laboratory staff and then assembled into the final data package. The assembled package was peer reviewed and checked to ensure that the DQOs were met, that the analyses met the program objectives, and that the data was traceable and defensible. The data was

also reviewed for compliance with the documented procedures and quality objectives in the work plan. Also, data was reviewed for internal consistency and against expected or known values.

After the final laboratory data package review, it was subjected to a formal audit. The audit process is coordinated by the QA Manager and follows the procedure outlined in the ICF Data Review SOP. The formal audit process included a 100-percent review of all hand-calculated values and a 20-percent review of computer-generated results. The process also checked the traceability of a final result through the instrument calibration and to the sample preparation steps. A formal report was issued to the facility supervisors at the completion of the audit for response. Upon completion of the responses, the auditor released the results to the Program Manager for review and reporting. The final laboratory data package and the audit report are maintained in the laboratory files.

2.2.1.17 Organics Quality Control Results

Laboratory QC samples were analyzed to assess precision and accuracy of the sample preparation and analytical procedures. The number and type of laboratory QC samples was based on the total number of field samples and as specified in ICF SOPs and the Field Sampling and Logistics Plan (Arthur D. Little, 2001). For this program, the following laboratory QC samples and measures were used to evaluate accuracy and precision of the analytical data: surrogate recoveries, procedural blanks, blank spike samples, laboratory duplicates, standard reference materials, and oil reference standards. The results for the organic QC samples and measures are presented in Appendix C, along with the results for the associated environmental samples. Discussion and interpretation of the results are provided in the following sections.

2.2.1.18 Surrogate Results

Surrogate compounds were added to all environmental and QC samples prior to sample preparation. These compounds were added to determine the efficiency of the sample extraction and analysis procedures. Surrogate recoveries were evaluated to assess analytical method accuracy relative to sample matrix and laboratory performance.

For the PAH analyses, all of the environmental and QC sample surrogate recoveries were within the recovery acceptance limits, with several exceptions. Surrogates acenaphthene-d10 and phenanthrene-d10 recovered low in sample N25-126-ACD; naphthalene-d8 recovered low in samples N25-128-ACD, PBS-75-BW, L14-90-AD, L14-96-FS, and BPS-109-FS; naphthalene-d8 and acenaphthene-d10 recovered low in sample L14-92-AD; and benzo(a)pyrene-d12 recovered low in samples SIS-12-HW and SIS-21-HW. Also, one blank spike had low recoveries for all four surrogates and the associated method blank had low recoveries for two surrogates. The surrogate recovery outliers ranged from 31 to 44 percent. The target compound results in the affected samples are considered to be estimated values.

For the pesticide/PCB analyses, all of the environmental and QC sample surrogate recoveries were within the recovery acceptance limits, with one exception. One blank

spike had a low recovery for dibromo-octafluoro-biphenyl. The method blank and associated field samples had acceptable surrogate recoveries indicating that the poor extraction efficiency in the blank spike was an isolated occurrence, thus, the low surrogate recovery in the blank spike do not adversely affect the quality or usability of the associated environmental sample data.

2.2.1.19 Procedural Blanks

A laboratory procedural blank was prepared with each sample preparation batch by extracting a blank sample matrix (sodium sulfate) as if it were one of the environmental samples. Procedural blanks are used to assess the potential of contamination introduced during sample preparation and analysis. PAH, pesticide, and PCB analyses were performed on each PB.

For the PAH analyses, between five and eighteen PAH target compounds were detected at trace concentration less than the MRL in each of the tissue PBs. For the pesticide and PCB analyses, between one and three pesticides and between one and ten PCBs were detected at trace concentrations less than the MRL in each of the tissue PBs. One pesticide/PCB PB associated with samples BPS-121-FS and N25-126-ACD was contaminated with the matrix spike solution at concentration above the MRL. Inadequate sample mass was available to re-prepare and re-analyze these two samples, and thus they have been reported, as is, along with the contaminated blank. There is no indication that the associated samples were contaminated with the matrix spike solution.

Environmental sample results that were within 5 times the associated PB concentration were qualified with a "B" to indicate that the compound was also present in the blank. Of the results that were qualified with a "B", none of these results were at concentrations greater than 5 times the sample-specific MRL. Results that were qualified with a "B" may be biased high or may be false positives.

2.2.1.20 Blank Spike Sample Recoveries

A blank spike sample was prepared with each sample preparation batch by spiking a blank sample matrix with known concentrations of a subset of the target compounds. BSs are used to assess the accuracy of the sample preparation and analysis procedures independent of sample matrix effects.

For the PAHs analyses, the recoveries of between three and sixteen compounds in each tissue BS exceeded the acceptance criteria with recoveries ranging from 126 to 160 percent. The QC sample results were adjusted based on surrogate recoveries and these exceedances may have resulted from lower surrogate recoveries in the BS analysis. The affected target compound results in the associated samples are considered to be estimated values and may be bias high.

For the pesticide/PCB analyses, all recovery criteria were met with several exceptions: 4,4'-DDD recovered high in four of eight BSs; 2,4'-DDT recovered high in three of eight BSs; 2,4'-DDE and dieldrin recovered high in two of eight BSs; and PCB 180 recovered high in one of eight BSs. The outlying BS recoveries ranged from 130-

140 percent. The affected target compound results in the associated samples are considered to be estimated values and may be bias high.

2.2.1.21 Laboratory Duplicates

Laboratory duplicates were prepared with each sample preparation batch by extracting a second separate aliquot of an environmental sample. Laboratory duplicates were evaluated to assess analytical precision related to laboratory performance and sample matrix. PAH, pesticide, and PCB analyses were performed on each laboratory duplicate.

For the PAH, pesticide, and PCB analyses, good laboratory duplicate precision was noted with one exception. Several compounds in the pesticide and PCB duplicate analysis of sample BPS-113-FS exceeded relative percent difference (RPD) criterion of less than 30 percent for all compounds detected at concentrations greater than 2 times the MRL. The results for this sample should be considered estimated due to potential sample heterogeneity. For the remaining field duplicate pairs, the RPDs were less than 30 percent for all of the compounds detected at concentrations above two times the MRL and for the majority of the compounds detected at concentrations below two times the MRL. The laboratory duplicate precision criterion does not apply to compounds detected below two times the MRL due to increased variability at low concentrations. (RPD was calculated as the absolute difference between the two measurements divided by the mean of the two measurements).

2.2.1.22 Standard Reference Materials

Instrument Standard Reference Materials (IRM) were analyzed with each instrumental analytical sequence to assess accuracy of the instrument calibration. A matrix-specific SRM was prepared and analyzed with each sample preparation batch to assess accuracy of the analytical method relative to sample preparation and analysis procedures. PAH, pesticide, and PCB analyses were performed on each SRM.

Instrument SRM (IRM)
IRM 1491 was analyzed prior to each PAH, pesticide, and PCB analytical sequence. The percent differences (%Ds) of the measured values versus the certified values were within 15 percent for all instrument SRMs with several exceptions. PCB 101 and PCB 128 recovered high in one IRM (15.4 and 17%, respectively) and 4,4'-DDD recovered low in two IRMs (-23.4 and -27.4%). The PCB 101 and PCB 128 results may be bias high in the associated samples and the 4,4'-DDD results may be bias low in the associated samples.

Tissue SRM
SRM 1974a was prepared and analyzed for PAHs, pesticides, and PCBs along with the tissue samples.

For the pesticide/PCB analyses, all of the compound concentrations were within 35 percent of the certified values with the following three exceptions: cis-Nonachlor recovered high in two of eight SRMs (66.7 and 57.9 percent) and dieldrin recovered

high in one SRM (54.2 percent). The affected target compound results in the associated samples are considered to be estimated values and may be bias high. For the PAH analyses, all of the compound concentrations were within 35 percent of the certified values with the following exceptions: anthracene recovered high in all SRMs (215, 37.5, 197, 118, 130, 149, and 138 percent); acenaphthylene recovered high in one SRM (155 percent); phenanthrene recovered high in one SRM (43.2 percent); benzo(b)fluoranthene recovered high in two SRMs (51.1 and 60.8 percent); benzo(k)fluoranthene recovered high in 3 SRMs (70.5, 53.1, and 58.1 percent); benzo(e)pyrene recovered high in two SRMs (38.1 and 35.7 percent); benzo(g,h,i)perylene recovered high in two SRMs (52.7 and 40.4 percent); and naphthalene recovered high in two SRMs and low in one (56.6, 82.1, and –47.2 percent).

The high recoveries of anthracene are consistent with the results obtained for this compound in multiple (more than 40 samples) analyses of SRM 1974a over the last four years by ICF. This QC issue does not impact the quality or usability of the associated sample data since acceptable recoveries for anthracene were noted in the IRM analyses, and since it appears that the certified value for anthracene in NIST SRM 1974a is incorrect. The results in the tissue samples for the remaining compounds may be biased high as indicated by the high recoveries in the tissue SRM. These SRM exceedances have a minor impact on the quality and usability of the associated sample data since the exceedances were not extreme and did not result in any data being considered unusable.

2.2.1.23 Control Oil Analyses

A North Slope crude oil sample was analyzed prior to each analytical sequence for PAHs. The results of the North Slope Crude oil analyses were used to evaluate accuracy of the analytical methods, provide a chromatographic pattern for comparisons with samples, and provide an independent check of the quantitation for alkyl PAHs. Results of the control oil analyses were compared to laboratory mean values generated from multiple analyses of the oils. All of the PAH results were within the acceptance limits.

Table 3. Data Quality Objectives – PAHs by GC/MS/SIM

Element or Sample Type	Minimum Frequency	Acceptance Criteria
MS Tuning Check	Prior to each analytical sequence	Using PFTBA: m/z 69: base peak abundance set to high sensitivity m/z 219: 30-60% of base peak abundance m/e 502: 2-8% of base peak
Initial Calibration	Prior to every batch sequence.	5 point curve; %RSD ≤25% for 90% of compounds and ≤35% for all compounds
Continuing Calibration	Every 12 field samples or 16 hours (whichever is more frequent) and at end of analytical sequence	%RSD ≤25% for 90% of compounds and %RSD ≤35% for all compounds.
Oil Reference Sample (North Slope Crude)	After each initial calibration sequence	%D ≤35% from laboratory established target value (use surrogate corrected results)
Instrumental SRM (SRM 1491)	After each initial calibration sequence	%D ≤15% from target value for all certified compounds (use surrogate corrected results)
Procedural Blank	Every preparation batch of 20 or fewer samples	No more than 2 compounds to exceed 5x target MDL unless compound not detected in associated sample(s) or associated sample compound concentration is >5x blank value
Standard Reference Material	Every preparation batch of 20 or fewer samples	%D ≤35% from target value for all certified compounds (use surrogate corrected results)
Blanks Spike	Every preparation batch of 20 or fewer samples	%R 35-125%; RPD ≤35%
Laboratory Duplicate	Every preparation batch of 20 or fewer samples	RPD ≤30% for all compounds at concentrations >2x MRL
Surrogate Compounds	Every sample	%R 35-125% for d_8-naphthalene and d_{12}-benzo[a]pyrene; 45-125% for d_{10}-acenaphthene and d_{10}-phenanthrene
Internal Standard Compounds	Every sample	Area response 50-200% of previous continuing calibration check standard

Table 4. Data Quality Objectives – Pesticides and PCB Congeners by GC/ECD

Element or Sample Type	Minimum Frequency	DQO/Acceptance Criteria
Initial Calibration	Prior to every instrument batch sequence or as needed indicated by continuing calibration check	5 point curve; %RSD ≤25% for 90% of compounds and ≤35% for all compounds
Continuing Calibration	After every 10 field samples or 18 hours, whichever is more frequent, and at end of instrument batch sequence	%RSD ≤25% for 90% of compounds and %RSD ≤35% for all compounds.
Instrumental SRM (SRM 1491)	After each initial calibration sequence	%D ≤15% from target value for all certified compounds (use surrogate corrected results)
Procedural Blank	One per preparation batch or one per 20 samples or one per extraction type	No compound to exceed the MRL
Blank Spike	One per 20 samples	Recovery between 45 and 125%
Laboratory Duplicate	Every preparation batch of 20 or fewer samples	RPD ≤30% for all compounds at concentrations >2x MRL
Standard Reference Materials	As requested (approximately 1 set per twenty field samples)	Percent difference within ± 35% for certified compounds
Surrogate Standards	Every sample and blank	Recovery between 45 and 125%
Internal Standard Compounds	Every sample	Area response 50-200% of previous continuing calibration check standard

Table 5. Organic Quality Control Result Summary – Polynuclear Aromatic Hydrocarbon Analyses

QC Sample or Measurement Type	Acceptance Criteria	Quality Control Result Summary	Impact to Data Quality and Usability
Initial Calibration	%RSD <25% for all compounds (up to 10% of compounds can be >25%, but <35%)	All criteria were met.	None.
Continuing Calibration	%D <25% for all compounds (up to 10% of compounds can be >25%, but <35%)	All criteria were met.	None.
Surrogate Recoveries	45 to 125% recovery (35 – 125% for d8-naphthalene) d8-naphthalene (d8n) d10-acenaphthene (d10a) d10-phenanthrene (d10p) d12-benzo(a)pyrene (d12b)	All criteria were met with the following exceptions: d10a and d10p recovered low in N25-126-ACD; d8n recovered low in N25-128-ACD, PBS-75-BW, L14-90-AD, L14-96-FS, and BPS-109-FS; d8n and d10a recovered low in L14-92-AD; and d12b recovered low in SIS-12-HW and SIS-21-HW. Low surrogate recoveries were also noted in one PB and BS.	Minor. The results for affected tissue samples should be considered estimated values due to low surrogate recoveries.
Procedural Blank (PB)	No compound to exceed 5 times the MDL unless sample amount is >10 times blank amount	All criteria were met. Several PAHs were detected in the tissue blanks at trace concentrations, but were less than 5 times the MDL.	Minor. Results within 5 times the blank result were qualified "B" and may be biased high or false positives.

Table 5. Organic Quality Control Result Summary – Polynuclear Aromatic Hydrocarbon Analyses Continued…

QC Sample or Measurement Type	Acceptance Criteria	Quality Control Result Summary	Impact to Data Quality and Usability
Blank Spike (BS) Sample Recoveries	35 to 125% recovery for spiked compounds	Each BS had 3 to 16 compounds with high spike recoveries ranging from 126 to 160%.	Minor. Results for these compounds in the associated samples may be bias high.
Laboratory Duplicate	RPD <30% for all compounds >2 times the MRL	All criteria were met.	None.
Instrument SRM (1491)	Measured values must be within 15% of true value for all certified compounds	All criteria were met.	None.
Tissue SRM (1974a)	Measured values must be within 30% of the true value on average for all compounds, not to exceed 35% of true value for more than 30% of the compounds	All criteria were met for the tissue SRM, with the exception of high responses for anthracene in 7 of 7 SRMs, acenaphthylene in 1 of 7, phenanthrene in 1 of 7, benzo(b) fluoranthene 2 of 7; benzo(k)fluoranthene in 3 of 7, benzo(e)pyrene in 2 of 7, and benzo(g,h,i)perylene in 2 of 7; and low responses for naphthalene in 3 of 7 SRMs.	Minor. The certified value for naphthalene and anthracene in SRM 1974a appears to be incorrect based on consistently high anthracene results in repeated analyses over the past four years. The results for the remaining compounds in the associated samples may be bias high.
Oil Reference Standard (North Slope Crude)	%D <35% for compounds above the RL	All criteria were met.	None.

Table 6. Organic Quality Control Result Summary – Pesticide and PCB Congener Analyses

QC Sample or Measurement Type	Acceptance Criteria	Quality Control Result Summary	Impact to Data Quality and Usability
Initial Calibration	5 point curve; %RSD ≤25% for 90% of compounds and ≤35% for all compounds	All criteria were met.	None.
Continuing Calibration	%RSD ≤25% for 90% of compounds and %RSD ≤35% for all compounds.	All criteria were met.	None.
Surrogate Recoveries	45 to 125% recovery	All criteria were met with the exception of one low surrogate recovery in blank spike sample.	None. Surrogate recoveries in all field samples were acceptable.
Procedural Blank	No compound to exceed the MRL	All criteria were met. Several pesticides and PCBs were detected at trace concentrations less than the MRL.	Minor. Results within 5 times the associated blank result were qualified with a "B" and may be biased high or may be false positives.
Blank Spike (BS) Sample Recoveries	45 to 125% recovery for spiked compounds	All criteria were met with several exceptions: 4,4'-DDD recovered high in 4 of 8 BSs; 2,4'-DDT recovered high in 3 of 8 BSs; 2,4'-DDE and dieldrin recovered high in 2 of 8 BSs; and PCB 180 recovered high in 1 of 8 BSs.	Minor. The 4,4'-DDD, 2,4'-DDT, 2,4'-DDE, dieldrin, PCB 180 results in the associated samples may be biased high by 30-40%.
Laboratory Duplicate	RPD <30% for all compounds >2 times the MRL	All criteria were met with the exception of several compounds in lab duplicate pair of sample 01-BPS-113-PHC-T-FS-DUP.	Minor. The results in sample 01-BPS-113-PHC-T-FS-DUP should be considered estimated due to potential sample heterogeneity.

Table 6. Organic Quality Control Result Summary – Pesticide and PCB Congener Analyses Continued…

QC Sample or Measurement Type	Acceptance Criteria	Quality Control Result Summary	Impact to Data Quality and Usability
Tissue SRM (1974a)	Measured values must be within 30% of the true value on average for all compounds, not to exceed 35% of true value for more than 30% of the compounds	All criteria were met with three exceptions: cis-Nonachlor recovered high in 2 of 8 SRMs and dieldrin recovered high in 1 of 8 SRMs.	Minor. The cis-Nonachlor and dieldrin results may be bias high in the associated samples.
Instrument Reference Material (IRM)	%D ≤15% from target value for all certified compounds	All criteria were met with several exceptions: PCB 101 and PCB 128 recovered high in one IRM; and 4,4'-DDD recovered low in two IRMs.	Minor. The PCB 101 and PCB 128 results may be bias high in the associated samples and the 4,4'-DDD results may be bias low in the associated samples.

2.2.2 Trace Metals Analysis in Fish Tissue

Prior to acid digestion, the homogenized tissue samples received from ICF were thawed and re-mixed with a Teflon stirring rod. The samples were then split into two portions, one subsample to be digested wet for Hg and the other to be freeze-dried and digested for determination of the remaining trace metals. The freeze-dried subsamples also provided the percent water content data to convert the Hg results from a wet-weight to dry-weight basis.

The concentrations of all metals (except Hg) were determined using 2 to 7 grams of wet-weight tissue weighed into 100-mL glass digestion flasks. These subsamples were freeze-dried, reweighed for percent water content, and then digested by the sequential addition of concentrated, high-purity nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl) with gentle refluxing. Aliquots of tissue Certified Reference Materials (CRMs) were digested along with the experimental samples. Once the tissue samples and CRMs were completely dissolved, the clear solutions were transferred to graduated cylinders, diluted to 20 mL with high-purity reagent water (18 megohm resistivity) rinses of the digestion flasks, and then stored in labeled 30-mL polyethylene screw-cap bottles for trace metal analysis.

Mercury determinations were carried out using 0.3 to 2 grams of wet tissue and dry CRMs weighed into 50-mL glass digestion tubes. These subsamples were digested by the addition of concentrated, high-purity HNO_3 and sulfuric acid (H_2SO_4) and

refluxing at 90°C for 1 hour in the Sealed tubes. The dissolved samples were transferred to graduated cylinders, diluted to 20 mL with high-purity reagent water rinses of the digestion tubes, and then stored in labeled 30-mL polyethylene screw-cap bottles.

Metal concentrations in the digested tissue samples, CRMs, and blanks were determined by flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption atomic spectrometry (GFAAS) with Zeeman or continuum background correction, cold-vapor atomic absorption spectrometry (CVAAS), or inductively coupled plasma-mass spectrometry (ICP-MS). The method used for each element and the corresponding MDLs are given in Table 10. All analytical techniques followed manufacturers' specifications and SOPs on file at FIT. These methods are based on EPA methods described for Series 7000 (FAAS and GFAAS), Series 7470 (CVAAS), and Series 6010A (ICP/MS) (EPA 1991).

2.2.2.1 Quality Control Measurements for Metal Analysis

For this project, QC measures included balance calibration, instrument calibration (FAAS, GFAAS, Zeeman Graphite Furnace Atomic Absorption Spectrometry [ZGFAAS], CVAAS and ICP/MS), matrix spike analysis for each metal, duplicate sample analysis, CRM analysis, procedural blank analysis and standard checks. With each batch of up to 40 samples, 2 procedural blanks, 2 CRMs, 2 duplicate samples and 2 matrix-spiked samples were analyzed.

Table 7. Summary of Instrumental Methods and Method Detection Limits for Metal Analysis of Fish Tissue

	Organisms	
Metal	**Method**	**MDLs (μg metal/g tissue dry weight)**
As – arsenic	ZGFAAS	0.03
Ba – barium	ICP-MS	0.01
Cd – cadmium	ICP-MS	0.001
Cr – chromium	GFAAS	0.01
Cu – copper	FAAS	0.7
Fe – iron	FAAS	2.5
Hg – mercury	CVAAS	0.001
Ni – nickel	GFAAS	0.01
Pb – lead	ICP-MS	0.003
Se – selenium	ZGFAAS	0.03
V – vanadium	GFAAS	0.01
Zn – zinc	FAAS	0.4

Notes:
CVAAS = Cold Vapor Atomic Absorption Spectrometry
FAAS = Flame Atomic Absorption Spectrometry
GFAAS = Graphite Furnace Atomic Absorption Spectrometry
ICP/MS = Inductively Coupled Plasma/Mass Spectrometry
MDL = Method Detection Limit
ZGFAAS = Zeeman Graphite Furnace Atomic Absorption Spectrometry

2.2.2.2 Instrument Calibration

Electronic balances used for weighing samples and reagents were calibrated prior to each use with certified (National Institute of Standards and Technology [NIST] traceable) standard weights. All pipets (electronic or manual) were calibrated prior to use. Each of the spectrometers used for metals analysis was initially standardized with a three- to five-point calibration with a linear correlation coefficient of r=0.999 required before experimental samples could be analyzed. Analysis of complete three- to five-point calibrations and/or single standard checks alternated every 5 to 10 samples until all of the analyses were complete. The RSD between complete calibration and standard check was required to be <15 percent or recalibration and reanalysis of the affected samples was performed.

2.2.2.3 Matrix Spike Analysis

Matrix spikes were prepared for a minimum of 5 percent of the total number of samples analyzed and included each metal to be determined. Results from matrix spike analysis using the method of standard additions provide information on the extent of any signal suppression or enhancement due to the sample matrix. If necessary (i.e., spike results outside 80 to 120 percent limit), spiking frequency was increased to 20 percent and a correction applied to the metal concentrations of the experimental samples.

2.2.2.4 Duplicate Sample Analysis

Duplicate samples from homogenized field samples (as distinct from field replicates) were prepared in the laboratory for a minimum of 5 percent of the total samples. These laboratory duplicates were included as part of each set of sample digestions and analyses and provided a measure of analytical precision.

2.2.2.5 Procedural Blank Analysis

Two procedural blanks were prepared with each set of 40 samples to monitor potential contamination resulting from laboratory reagents, glassware, and processing procedures. These blanks were processed using the same analytical scheme, reagents, and handling techniques as used for the experimental samples.

2.2.2.6 CRM Analysis

A common method used to evaluate the accuracy of environmental data is to analyze CRMs, samples for which consensus or "accepted" analyte concentrations exist. The following CRMs were used: Dogfish Muscle DORM-2; Lobster Hepatopancreas TORT-2; and Riverine Water SLRS-3, all certified by the National Research Council of Canada (NRC). Metal concentrations obtained for the CRMs were required to be within 20 percent of accepted values for >85 percent of the analyses. When no certified value for a metal was available in a tissue CRM (Ba), the Riverine CRM and tissue matrix spikes were used to evaluate analytical accuracy.

2.2.3 Bile FAC's (*Fluorescing Aromatic Compounds)*

This assay can demonstrate very recent exposure to metabolized organic compounds, e.g., PAHs. Bile samples were analyzed for metabolites of aromatic compounds using a high-performance liquid chromatographic procedure (Krahn et al., 1982, 1984) with fluorescence detection. Briefly, the (thawed) bile is injected directly into a high performance liquid chromatograph (HPLC) equipped with a Perkin-Elmer HC-ODS (reverse-phase) analytical column and two fluorescence detectors connected in series, and a gradient (100% water containing 5μL acetic acid/L, to 100% methanol). The excitation/emission wavelengths of one detector are set to 255/380 nm, where metabolites of phenanthrene (PHN) fluoresce. The excitation/emission wavelengths of the other detector are set to 380/430 nm, where the metabolites of benzo[*a*]pyrene (BaP) fluoresce. The total integrated area for each detector is then converted (normalized) to equivalents of known concentrations of either PHN or BaP standards. Quality assurance procedures include PHN and BaP calibration standards, a "bile pool" reference material, blank analyses, and replicate analyses to evaluate HPLC/UV fluorescence performance.

2.2.4 Cytochrome P4501A (CYP1A)

Cytochrome P4501A (CYP1A) is detected by a highly sensitive immunohistochemical (IHC) analysis that determines levels of CYP1A proteins in organs and tissues of interest. This data represents induction of the P4501A enzyme pathway by exposure to xenobiotic compounds (organics) and is used as an indicator of exposure to and pathways of contaminants to organisms.

An abbreviated method for the immunohistochemical analyses is as follows. Preserved tissues were placed in cassettes in 10% neutral buffered formalin, embedded in paraffin, and analyzed immunohistochemically for the presence of CYP1A. Tissue sections (5 □m) mounted on Superfrost Plus slides (Fisher) were deparaffinated and hydrated as before (Smolowitz et al., 1991). Matching serial sections were incubated with 150 □l of 1-12-3p6 monoclonal antibody against scup CYP1A, using modifications of Smolowitz (Smolowitz et al., 1991). Formalin-fixed tissues were embedded in paraffin, and 5-□m sections were mounted on Superfrost Plus slides (Fisher) and analyzed immunohistochemically for the presence of CYP1A as before (Smolowitz et al., 1991). Matching serial sections were incubated using the ShandonTM coverslip system for 2 h with two 150-□l aliquots of MAb 1-12-3p6 or with nonspecific purified mouse myeloma protein (UPC-10, IgG2A, Organon Teknika, West Chester, PA), each at 1.5 □g/ml in 1% BSA/TBS added at 0 and 60 minutes. Blocking solutions, secondary antibodies, linker and color developer were components of the Signet (Medford, MA) murine immunoperoxidase kit. Color development was achieved as described before using 2% 3-amino-9-ethylcarbazole and 1% hydrogen peroxide. Sections were counterstained with Mayer's hematoxylin. Slides were examined with a Zeiss Axioskop microscope and relative staining intensities were determined subjectively by comparing the staining of samples to that of control and highly induced 3,3',4,4' tetrachlorobiphenyl-treated scup liver sections included in each run. Nonspecific staining, if present, was determined by comparison with UPC-10 stained sections. Staining occurrence was scored as 0-no staining (or

equal to UPC staining), 1-rare- few cells staining, 2-many cells staining, 3-multifocal and diffuse-all cells staining. The intensity of staining was scored as 0-none (or equal to UPC staining), 1-mild, 2-moderate, 3- medium, 4-strong, 5-very strong. A scaled product of staining occurrence times the staining intensity was determined for each cell type. Therefore, IHC scores (being the product of 2 numbers, the first from 0-3, and the second from 0-5) could range from 0 to 15.

Quality assurance included the following steps:

1. Internal standards were included in each staining run to assure the consistency and quality of a run, and to determine maximum (occurrence 3 X intensity 5=15) and minimum (0) staining.

2. All tissues were stained with UPC 10 to determine if nonspecific staining was present.

3. As part of the standard Signet protocol, slides were presoaked in 3% H_2O_2 to eliminate endogenous peroxidase activity.

4. Any slides with questionable staining were re-run.

2.2.5 Tissue Analysis Strategy

Whole body analyses for organic and metal tissue contaminant residues (note that these fish had small pieces of tissues and organs removed for the Bile FAC analyses and the CYP1A analyses). This was done so that the results of the assays could be compared from individual fish, rather than using separate fish for individual assays. However, some small (less than 20 g) Cod were collected from the project sites. These fish were too small to conduct all of the analyses on individual fish. For these small Cod, a subset was retained for whole body tissue contaminant residue analyses, and a second subset was retained for whole body CYP1A analyses. These fish were too small to collect bile for the bile FAC analyses.

Bile FAC analyses were conducted on larger fish, when bile could be sampled from the gall bladder.

Cytochrome P4501A (CYP1A) induction was assayed on multiple organs from each fish. These organs included: gill, heart, liver, kidney, gonad, gut, muscle and skin.

Morphometric data was acquired for each dissected fish. This data included: length, weight, sex, and observations about parasite load and other pathologies (See dissection methods and fish dissection sheets in appendices).

2.2.6 Statistical Analyses

Data conditioning
The analytical results with qualifiers were treated as follows:

1. Included data:

- All values without qualifiers from the analytical laboratories.
- All values with the "b" qualifier, i.e., result detected in the associated procedural blank and sample result is less than 5 times the result found in the procedural blank.
- All values with the "j" qualifier--estimated result detected below the adjusted minimum reporting limit.

2. Excluded data:

- Napthalene parent compound, due to high concentrations in system blank.
- Benzo[g,h,i]perylene, due to high concentrations in system blank.

Data entry, normalizations and transformations
(All statistical analyses were performed on data that was entered into Excel spreadsheets and checked for errors after entry and before any manipulations).

Several steps were taken to improve the power of statistical analyses. First, to control for variation in measured trace organic compound concentrations caused by lipid content (Hebert and Keenleyside, 1995), wet-weight, concentrations reported from the analytical laboratory were normalized to the lipid content, as follows:

Lipid-weight concentration = measured wet-weight concentration ÷ lipid decimal %

This provided lipid-normalized concentrations for statistical analyses. Dry-weight trace metal concentrations, bile fluorescence values and P4501A values were not normalized to lipid. Second, because the variance was proportional to the mean for most variables, the data were transformed to ensure conformance to the assumptions of parametric statistical procedures using the log transformation, as follows:

Transformed value = $\log_{10}$(value)

For variables that included zeros (i.e., endosulfans, endrins, P4501A values in liver hepatocytes and gut mucous epithelia cells), one was added to each value.

Statistical Procedures
Several statistical procedures were performed to explore patterns in the data. First, a one-way analysis of variance (ANOVA) was performed for each fish species to examine the effects of site, with weight as a covariate, on the concentrations of organic analytes and trace metals, as well as bile fluorescence and P4501A induction in two tissues. A separate two-way ANOVA with interactions was performed to determine whether there were significant interactions between the effects of site and weight for any contaminant. Organic analytes were analyzed as totals for all PAHs,

for low-molecular-weight PAHs (two and three ring compounds), high-molecular-weight PAHs (four, five and six ring compounds), all PCBs, and all pesticides, as well as the following pesticide groups:

- all DDTs = 2,4'-DDD, 2,4'-DDE, 2,4'-DDT, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT
- all Chlordanes = alpha-Chlordane, gamma-Chlordane, cis-Nonachlor, trans-Nonachlor, Heptachlor, Heptachlor Epoxide, Oxychlordane, Methoxychlor
- all HCHs = alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane, delta-hexachlorocyclohexane
- all endrins = Endrin, Endrin Aldehyde, Endrin Ketone
- all endosulphans = Endosulfan I, Endosulfan II, Endosulfan Sulfate.

Second, backward stepwise multiple regressions were used to assess the effects of total low-molecular-weight PAHs, total high-molecular-weight PAHs, total PCBs, and total pesticides on P4501A activity. Transformed data were used for the regression analyses. Third, clustering was performed using Ward's minimum variance method on standardized species-site means for all organic analytes, all PAHs, all PCBs, and all pesticides. All statistical analyses were performed with the JMP software package (SAS Institute, 2000).

Statistical power of our analyses was tested in two ways. In both cases, data for the Four Horn Sculpin were used. The first test examined the power of this monitoring program to detect differences between sites within a sampling period. This procedure revealed how the percentage difference that could be detected between two sites varies according to the number of fish collected from each of four sites (Sokal & Rohlf, 1995). Because this test is based on the Coefficient of Variation (CV), an average CV was calculated from sites where more than two Four Horn Sculpin were collected (i.e., Bullen Point, Point Brower and Stump Island). The second test examined the percentage change at a site that could be detected through time (i.e., trend analysis) if 20 fish were collected in each year (Gerrodette, 1987). This analysis assumes that the rate of change is consistent through time.

3.0 Results

3.1 Collections

The number of fish of each species captured at the five sampling sites in 2001 is provided in Table 8.

Table 8. Summary of fish collected in the summer of 2001. Species in bold type were selected for chemical and biomarker analyses.

Species	Stump Island	Point Brower	Liberty	Bullen Point	Northstar	Species Total
Dolly Varden	4	1		3		8
Arctic Cisco	11	5	8	9		33
Humpback Whitefish	13	3				16
Arctic flounder	8	7				15
Four Horn Sculpin	12	5	2	10		29
Broad Whitefish		6				6
Arctic Cod			8		6 + composite	15
Snailfish				2		2
Total per site	48	27	18	25	7	
					Grand Total	124

It can be seen from Table 8 that a total of 124 fish representing 8 species were collected and processed for analysis. Eighty-nine of these were eventually analyzed. A map of the collection sites is provided in Figure 1.

3.2 Trace Organic Substances in Fish Tissues

In Table 9, all of the results of statistical analyses to determine if there are site or weight differences in any of the independent variables are provided.

Table 9. Results of one-way ANOVA for the effects of site, with weight as a covariate, on the concentrations of trace organic chemicals in five species of fish. Statistically significant results ($p \leq 0.05$) are indicated by bold type.

		Weight	Site		Interaction
Analyte Group & Species	r^2	p	p	Student's[a,b]	p
Total PAHs					
Arctic Cisco	0.1712	0.1826	0.3553	PB=L=SI=BP	0.1957
Arctic Cod	0.1537	0.4515	0.1977	L=NS	0.8888
Broad Whitefish	0.3312	0.3100	-	PB	-
Four Horn Sculpin	0.1827	0.1582	0.7534	PB=BP=L=SI	0.7527
Humpback Whitefish	0.3129	0.7709	0.0549	SI=PB	0.0776
Low-molecular-weight PAHs					
Arctic Cisco	0.1475	0.2412	0.4059	PB=L=SI=BP	0.1728
Arctic Cod	0.1980	0.4190	0.1342	L=NS	0.6095
Broad Whitefish	0.2221	0.3454	-	PB	-
Four Horn Sculpin	0.2850	0.1433	0.3525	PB=BP=L=SI	0.7229
Humpback Whitefish	0.3218	0.9466	0.0639	SI=PB	0.3607

		Weight	Site		Interaction
Analyte Group & Species	r^2	*p*	*p*	Student's[a,b]	*p*
High-molecular-weight PAHs					
Arctic Cisco	0.5401	**0.0004**	**0.0168**	PB>SI=L=BP	0.7825
Arctic Cod	0.0238	0.7317	0.6442	L=NS	**0.0457**
Broad Whitefish	0.2331	0.3321	-	PB	-
Four Horn Sculpin	0.4885	0.3661	**0.0009**	PB=SI>BP, PB=SI=L, L=BP	0.8324
Humpback Whitefish	0.0453	0.5359	0.9890	PB=SI	**0.0197**
Total PCBs					
Arctic Cisco	0.3479	0.1017	**0.0310**	SI>BP=L, SI=PB, PB=BP=L	**0.0092**
Arctic Cod	0.1787	0.4678	0.3273	NS=L	0.6423
Broad Whitefish	0.0007	0.9604	-	PB	-
Four Horn Sculpin	0.2014	0.2726	0.1861	BP=SI=PB=L	0.7426
Humpback Whitefish	0.4134	0.5316	0.0695	SI=PB	0.7626
Total Pesticides					
Arctic Cisco	0.4722	**0.0067**	**0.0157**	PB=SI>L=BP	0.7180
Arctic Cod	0.3465	**0.0444**	0.9788	NS=L	0.3431
Broad Whitefish	0.2949	0.2655	-	PB	-
Four Horn Sculpin	0.2479	0.1600	0.0929	PB=SI=L=BP	0.6448
Humpback Whitefish	0.5550	0.1490	0.0607	SI=PB	0.8332
Total Chlordanes					
Arctic Cisco	0.5988	**0.0052**	**0.0003**	SI=PB>L=BP	0.6746
Arctic Cod	0.3465	**0.0406**	0.8919	NS=L	0.2481
Broad Whitefish	0.2695	0.2912	-	PB	-
Four Horn Sculpin	0.2957	0.1527	**0.0441**	SI>BP, SI=PB=L, PB=L=BP	0.7418
Humpback Whitefish	0.7256	**0.0361**	**0.0126**	SI>PB	0.7122
Total DDTs					
Arctic Cisco	0.3078	0.1767	**0.0492**	SI>BP=L, SI=PB, PB=BP=L	**0.0083**
Arctic Cod	0.0912	0.6624	0.4709	NS=L	0.6047
Broad Whitefish	0.1497	0.5200	-	PB	-
Four Horn Sculpin	0.1867	0.0668	0.4864	L=SI=PB=BP	**0.0292**
Humpback Whitefish	0.3268	0.2815	0.2826	SI=PB	0.8874
Total HCHs					
Arctic Cisco	0.3473	0.4365	**0.0071**	BP=PB=L>SI	0.5865
Arctic Cod	0.3335	0.8613	**0.0447**	NS>L	0.7163
Broad Whitefish	0.2264	0.3402	-	PB	-
Four Horn Sculpin	0.2189	0.5454	0.1659	L=SI=PB=BP	**0.0253**
Humpback Whitefish	0.0687	0.6398	0.3494	PB=SI	0.4192

		Weight	Site		Interaction
Analyte Group & Species	r^2	*p*	*p*	Student's[a,b]	*p*
Total Endosulfans					
Arctic Cisco	0.3823	0.7870	**0.0042**	SI>BP=L, SI=PB, PB=BP=L	0.8872
Arctic Cod	0.1318	0.7570	0.2246	L=NS	0.3786
Broad Whitefish	0.2167	0.3521	-	PB	-
Four Horn Sculpin	0.1990	0.5843	0.1532	PB=SI=BP=L	0.5393
Humpback Whitefish	0.0923	0.4394	0.2744	SI=PB	0.5773
Total Endrins					
Arctic Cisco	0.2200	0.7269	0.0716	SI=BP=L=PB	0.3140
Arctic Cod	-	-	-	Not Detected	-
Broad Whitefish	0.2137	0.3560	-	PB	-
Four Horn Sculpin	0.2021	**0.0443**	0.2948	SI=PB=BP=L	0.8548
Humpback Whitefish	0.5058	0.2069	**0.0038**	SI>PB	0.3474
Liver Hepatocyte P4501A					
Arctic Cisco	0.2187	0.8518	0.0889	L=PB=SI=BP	0.7255
Arctic Cod	0.4543	0.8514	**0.0141**	NS>L	0.9982
Broad Whitefish	0.2370	0.3275	**-**	PB	-
Four Horn Sculpin	0.3844	0.1875	**0.0090**	PB=SI>BP, PB=SI=L, BP=L	0.4735
Humpback Whitefish	0.0974	0.3482	0.2811	SI=PB	0.4951
Gut Mucus Epithelium P4501A					
Arctic Cod	0.4140	0.1230	0.2124	NS=L	0.6654
Four Horn Sculpin	0.2880	0.1856	0.1823	PB=BP=L	0.1567
PHN Equivalents					
Arctic Cisco	0.7871	**0.0018**	0.2360	BP=SI=PB	0.7073
Broad Whitefish	0.5860	0.1314	-	PB	-
Four Horn Sculpin	0.7296	0.5110	**<0.0001**	BP=SI=L>PB	0.4155
Humpback Whitefish	0.1453	0.2257	0.1910	PB=SI	0.8152
BaP Equivalents					
Arctic Cisco	0.9786	**0.0092**	**<0.0001**	BP>SI=PB	0.5985
Broad Whitefish	0.6219	0.1129	-	PB	-
Four Horn Sculpin	0.5440	0.6074	**0.0031**	SI>PB, SI=BP=L, L=PB	0.3586
Humpback Whitefish	0.2785	0.3663	0.3065	SI=PB	0.7133

[a] Student's *a posteriori* results are for Student's t test for least significant means. Values are arranged with the highest means on the left and the lowest on the right.
[b] BP = Bullen Point, L = Liberty, NS = Northstar, PB = Point Brower, SI = Stump Island.

3.2.1 PAH in Fish Tissues

The mean whole-body concentrations for total PAH ranged from less than 100 ng/g to over 1000 ng/g (lipid-normalized wet-weight). There was no significant effect of site on the concentrations of total PAHs in any of the fish species (Table 12, Figure 2), nor was there an effect of weight. There also were no significant interactions between weight and site for PAH concentrations. Where there were more than one species analyzed at a site, the Four Horn Sculpin had the highest concentrations of total PAH (Figure 2).

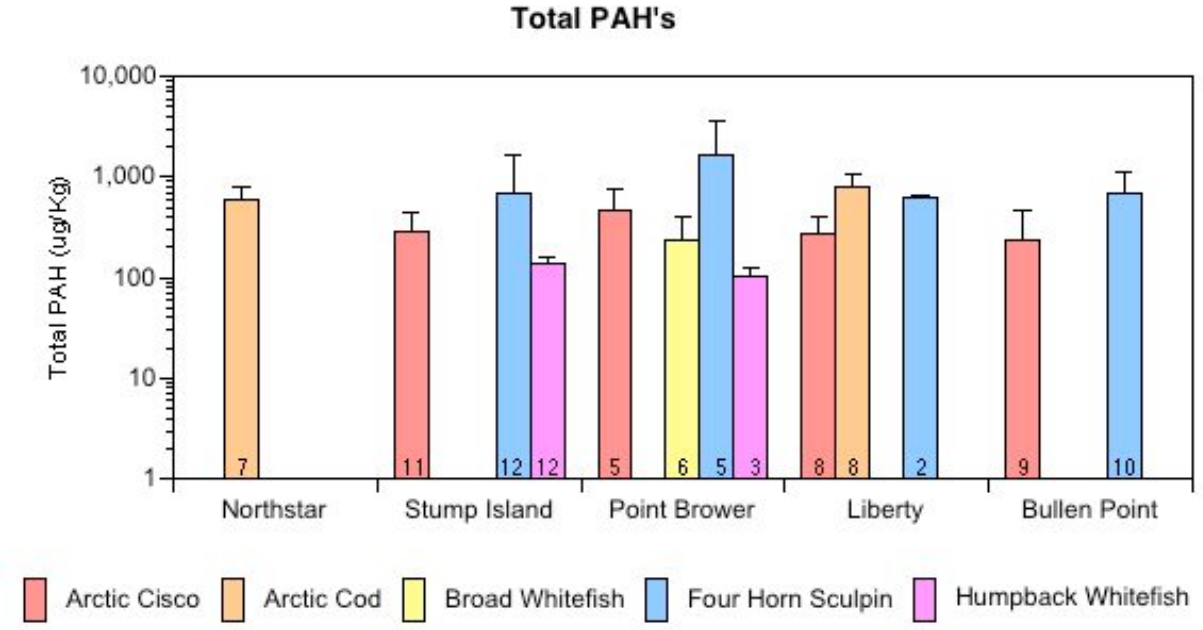

Figure 2. Total PAHs (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

In addition to the analyses of total PAHs, we divided the PAHs into two groups: low and high-molecularweight PAHs. The low-molecular-weight PAHs included the two and three-ringed compounds: the alkyl naphthalenes, phenanthrene and anthracene. The high-molecular-weight PAHs included all the 4 and 5-ringed compounds. For low-molecular-weight PAHs, there were no significant site differences for any of the species of fish (Table 9, Figure 3). For the high-molecular-weight PAHs, two species showed an effect of site: Arctic Cisco ($p= 0.0168$) and Four Horn Sculpin ($p= 0.0009$) (Table 9, Figure 4). For the Cisco, Point Brower had higher values than the other sites. For the Four Horn Sculpin, both Point Brower and Stump Island had higher concentrations than Bullen Point. For Arctic Cod and Humpback Whitefish there were significant interactions between site and weight that appear to be due to catching different sized fish at different stations, although neither were highly significant (e.g., $p >0.01$). For this group of PAHs the highest values were for Four Horn Sculpin.

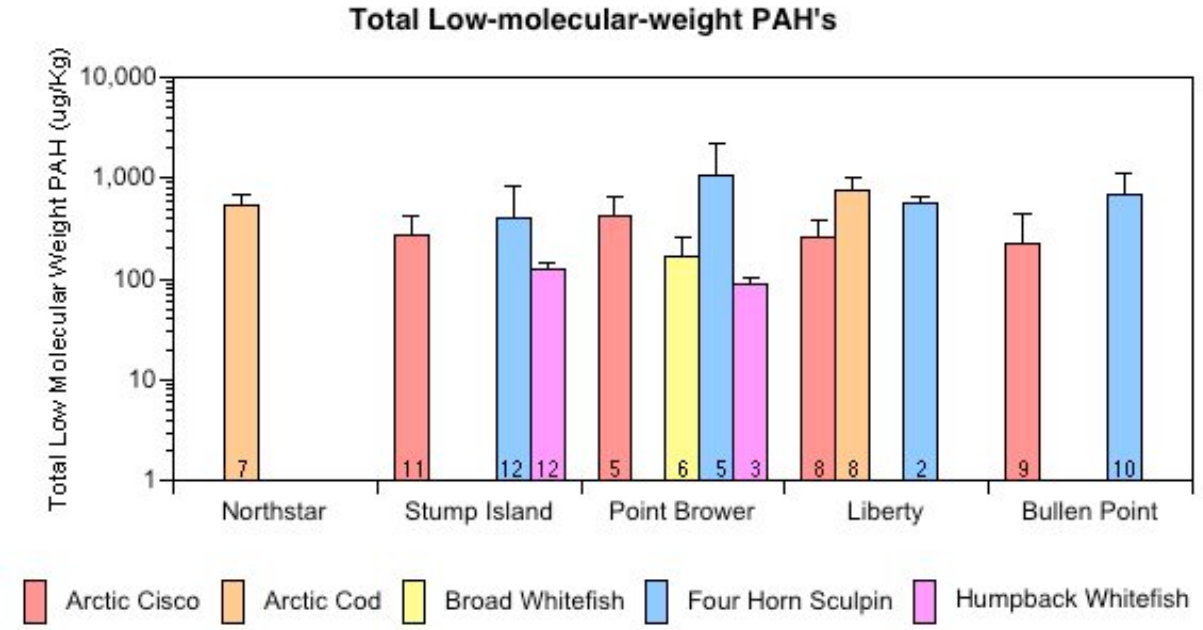

Figure 3. Total low-molecular-weight PAHs (lipid-normalized wet weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

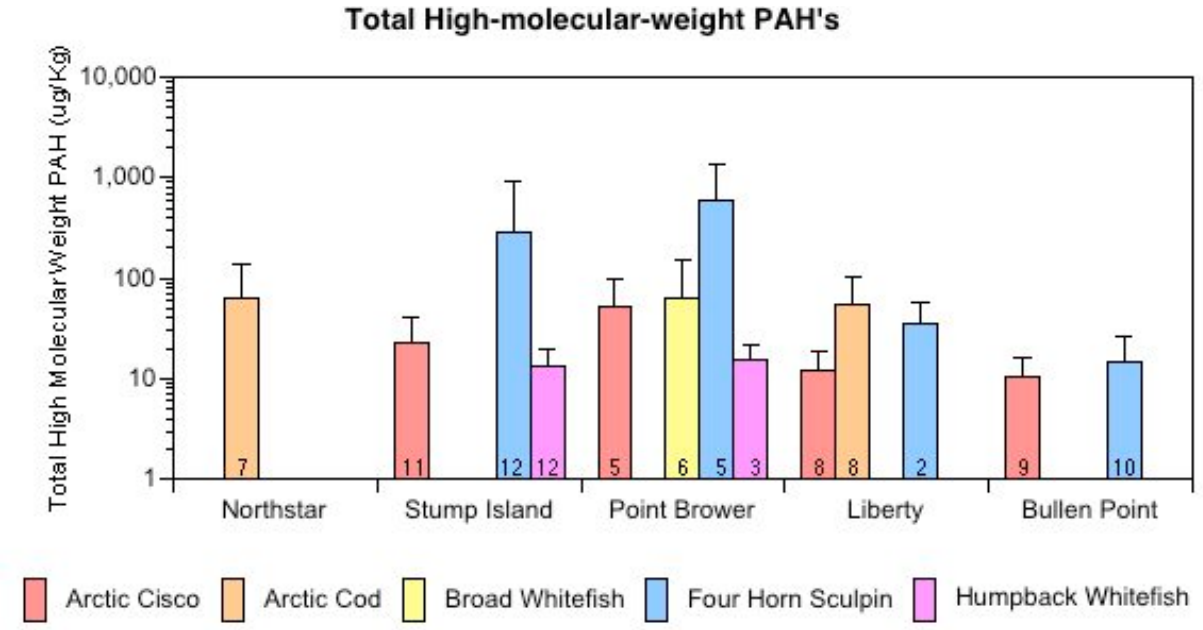

Figure 4. Total-high-molecular-weight PAHs (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

3.2.2 PCBs in Fish Tissues

The results of the analyses of PCBs were generally similar to those for PAHs. The only significant effect of site on the concentrations of PCBs was in Arctic Cisco (Table 12, Figure 5), in which Stump Island was higher than Bullen Point and Liberty. There were no effects of weight on PCB concentrations for any species. There was one significant interaction between weight and site for PCB concentrations, again with Artic Cisco. An analysis of weight distributions indicated two very large males, one from Stump Island and one form Point Brower, were outliers, and re-analysis without these two individuals eliminated the significant interaction. A few individual Four Horn Sculpin from Bullen Point had the highest PCBs of any of the fish analyzed in this study. This is also the location of an old Military installation, a Distant Early Warning (DEW) line site. There were 63 DEW line sites in the Arctic, in Greenland, Canada and Alaska and there were about 30 tons of PCBs estimated to be at these sites (AMAP, 1997).

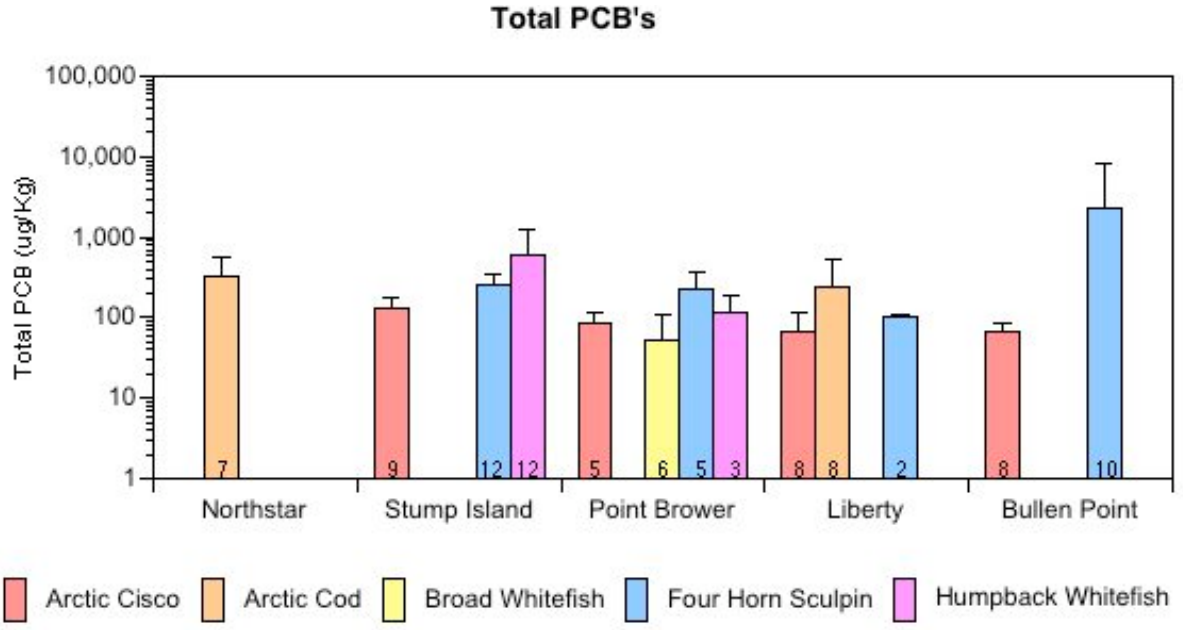

Figure 5. Total PCBs (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

There are two patterns of relative congener abundance in the fish from this study. The first is a mixture dominated by high-molecular-weight congeners (e.g., IUPAC congener 153) that is the most commonly encountered in our samples (Figure 6). A second pattern includes a similar congener composition of high-molecular-weight compounds, but also has significant, and sometimes dominant, low-molecular-weight components (e.g. IUPAC congener 8) (Figure 7).

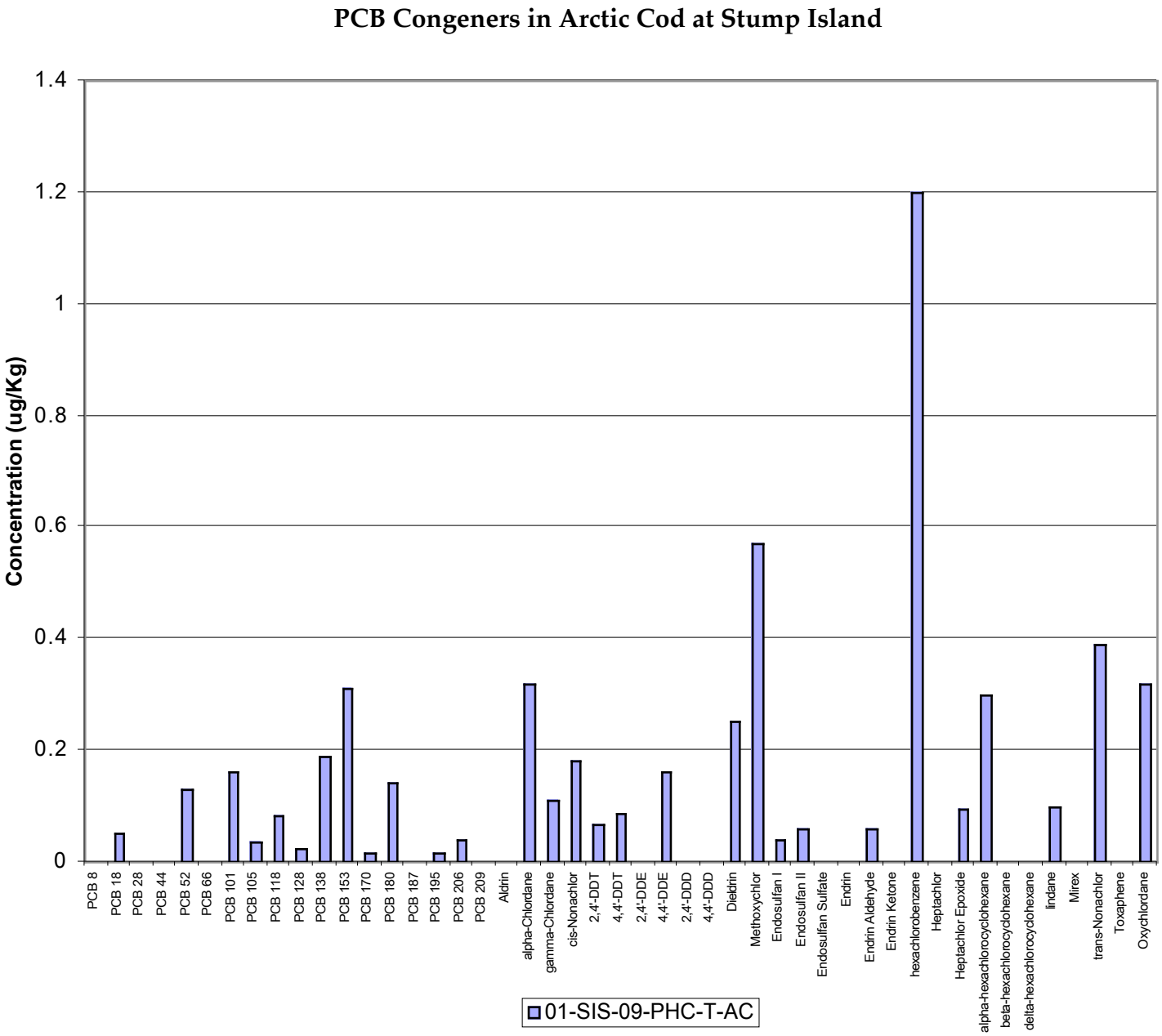

Figure 6. The pattern of PCB congeners in an Arctic Cod captured at Stump Island.

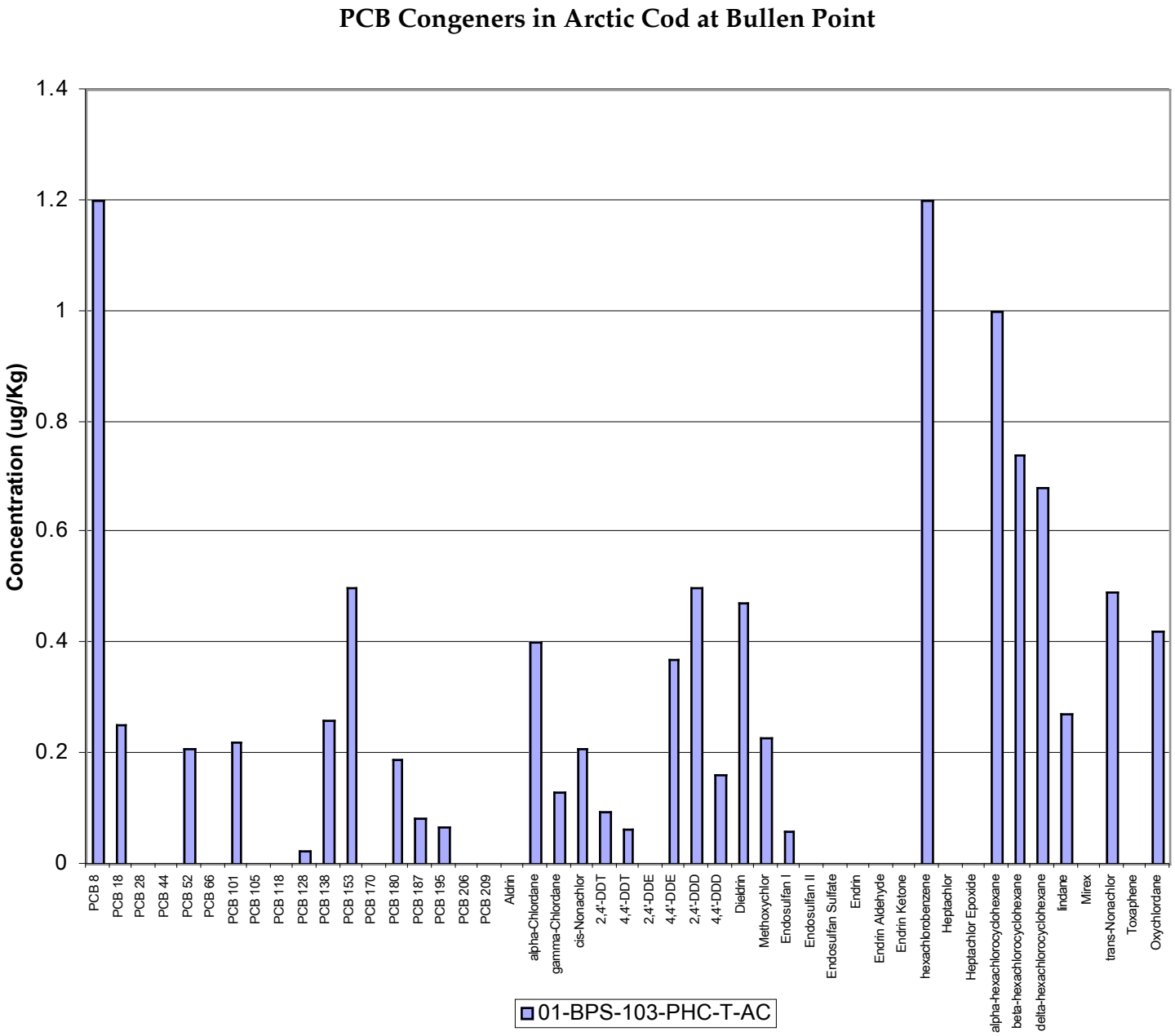

Figure 7. The pattern of PCB congeners in an Arctic Cod captured at Bullen Point.

3.2.3 Pesticides in Fish

Arctic Cisco was the only fish species that showed significant variation in total pesticide concentrations among sites (Table 12, Figure 8). Arctic Cisco at Point Brower and Stump Island had significantly higher total pesticide concentrations than those Liberty or Bullen Point. There was a significant effect of weight on pesticide concentrations in Arctic Cisco and Arctic Cod, but there were no significant interactions between weight and site (Table 9). In addition to total pesticides, we statistically analyzed a variety of separate pesticide groups that made up the total pesticide category: Chlordanes, DDTs, hexachlorohexanes, Endosulfans, and Endrins.

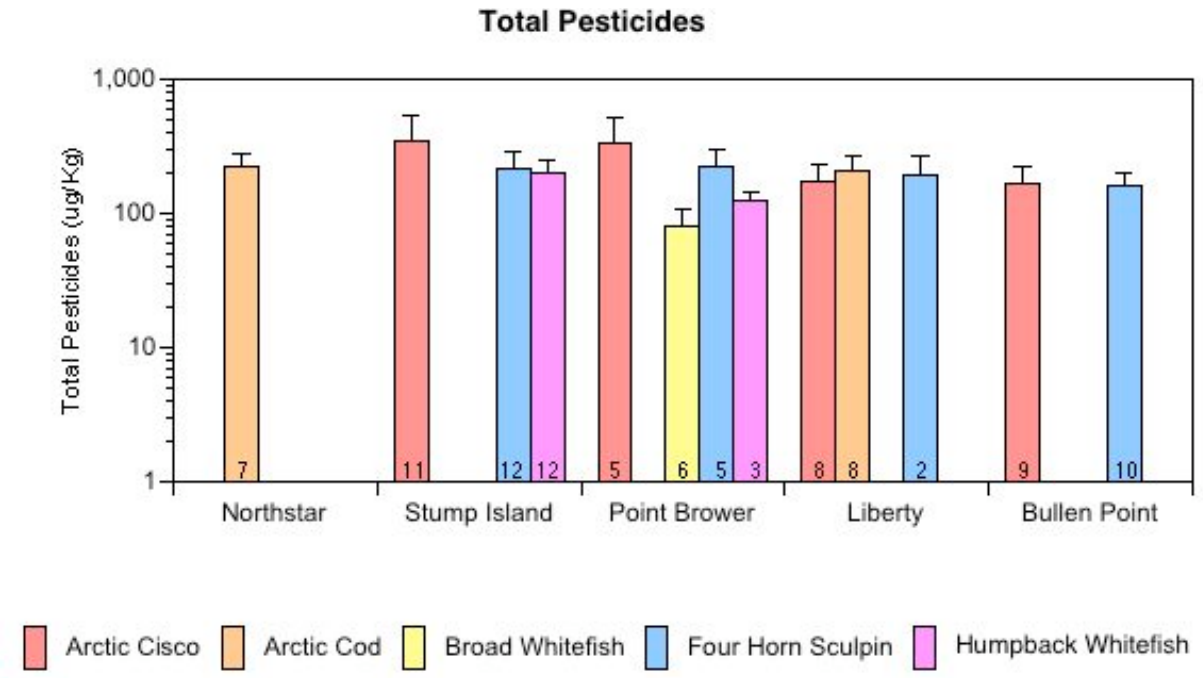

Figure 8. Total Pesticides (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

For chlordanes, three species showed significant variation with site: Arctic Cisco, Four Horn Sculpin and Humpback Whitefish (Table 12, Figure 9). For the Artic Cisco, Stump Island and Point Brower had equivalent concentrations and these were higher than the other sites at which this species was caught. For the Four Horn Sculpin, Stump Island had higher concentrations than did Bullen Point. For the Humpback Whitefish, Stump Island had higher concentrations than Point Brower.

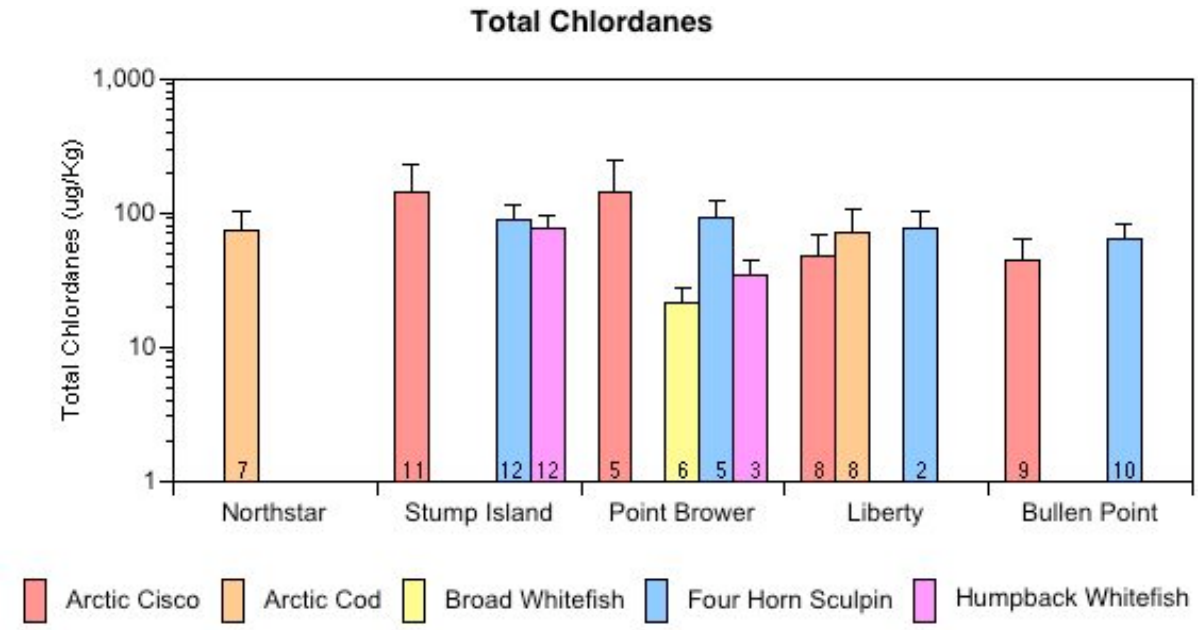

Figure 9. Total chlordanes (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

For DDTs, Arctic Cisco showed significant variation between collection sites, with Stump Island having higher concentrations than Bullen Point and Liberty (Table 12, Figure 10). There were significant interactions between weight and site for Arctic Cisco and Four Horn Sculpin. The high significance (p=0.0185) of the interaction for Arctic Cisco was substantially reduced when the two large fish at Stump Island and Point Brower were removed from the analysis, as was discussed for PCBs.

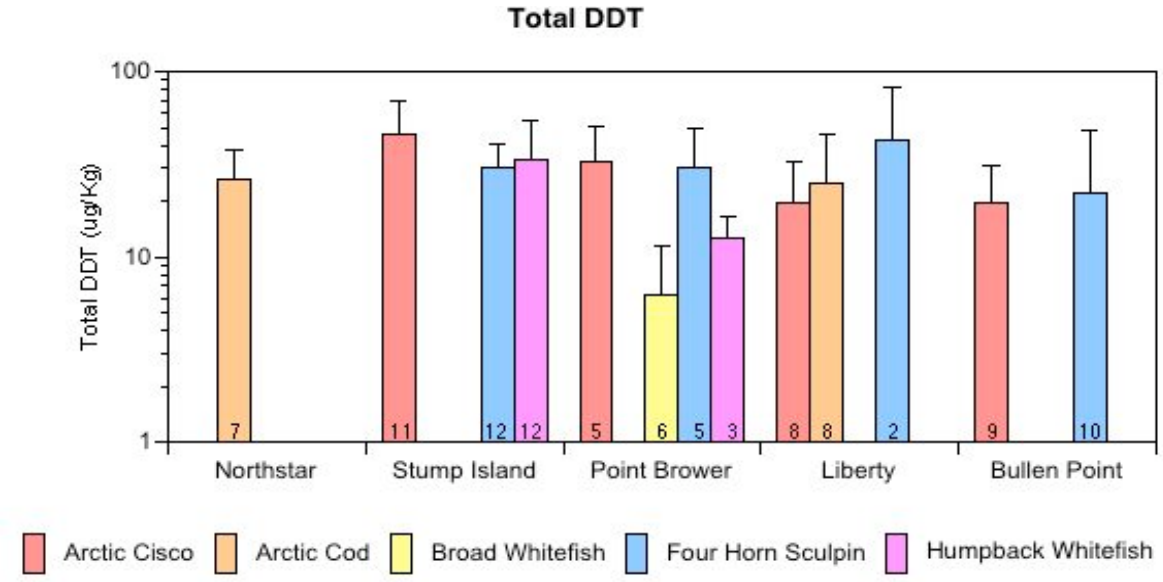

Figure 10. Total DDTs (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

For the hexachlorahexanes, Arctic Cisco (p=0.0071) and Arctic Cod (p=0.0447) showed significant variation with site. Arctic Cisco had similar whole-body concentrations at three stations that were all higher than at Stump Island. Arctic Cod were significantly higher at Northstar than at Liberty. There was a significant interaction of site and weight for Four Horn Sculpin (Table 12, Figure 11).

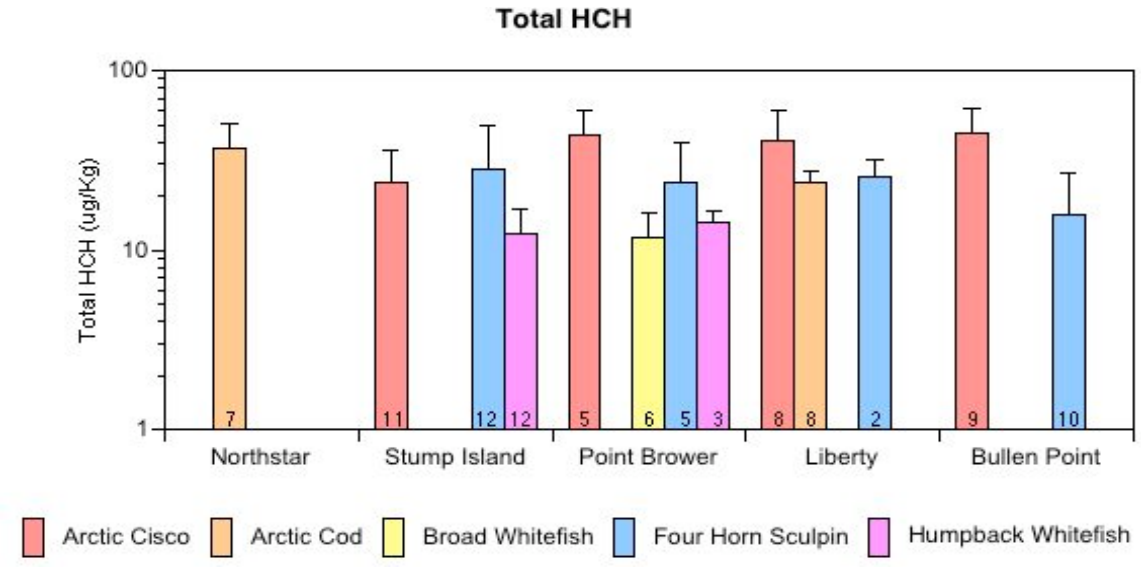

Figure 11. Total HCH (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

For endosulfans, only Arctic Cisco showed a significant variation with site. Stump Island had higher concentrations than Bullen Point or Liberty (Table 12, Figure 12).

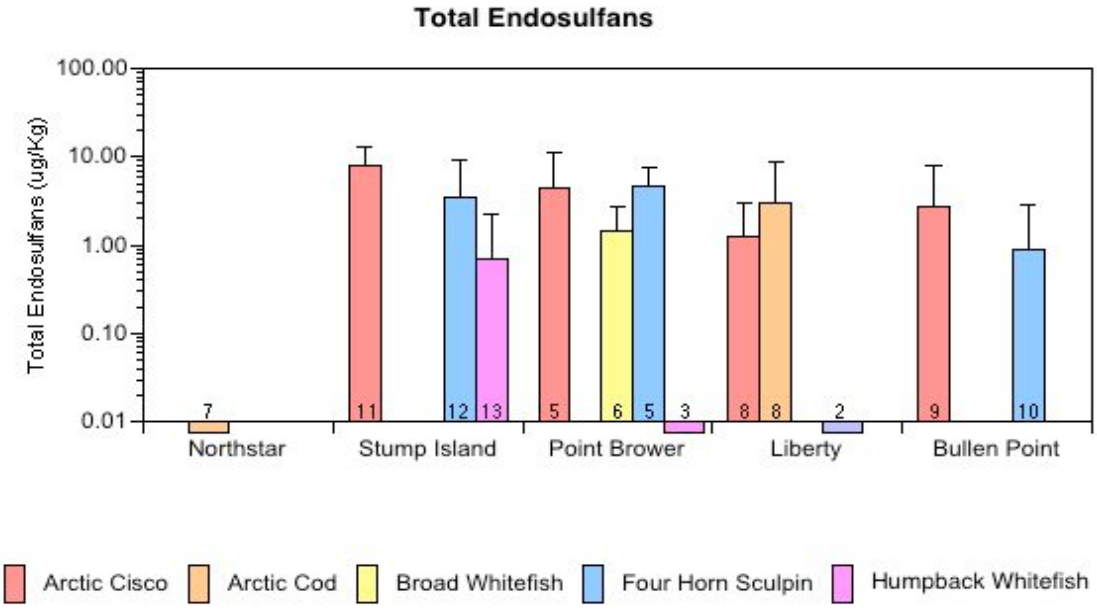

Figure 12. Total endosulphans (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar. Values below the x-axis are between 0 and the lowest value on the y-axis.

For endrins, Humpback Whitefish had significant variation due to site, with Stump Island having higher concentrations than Bullen Point (Table 12, Figure 13).

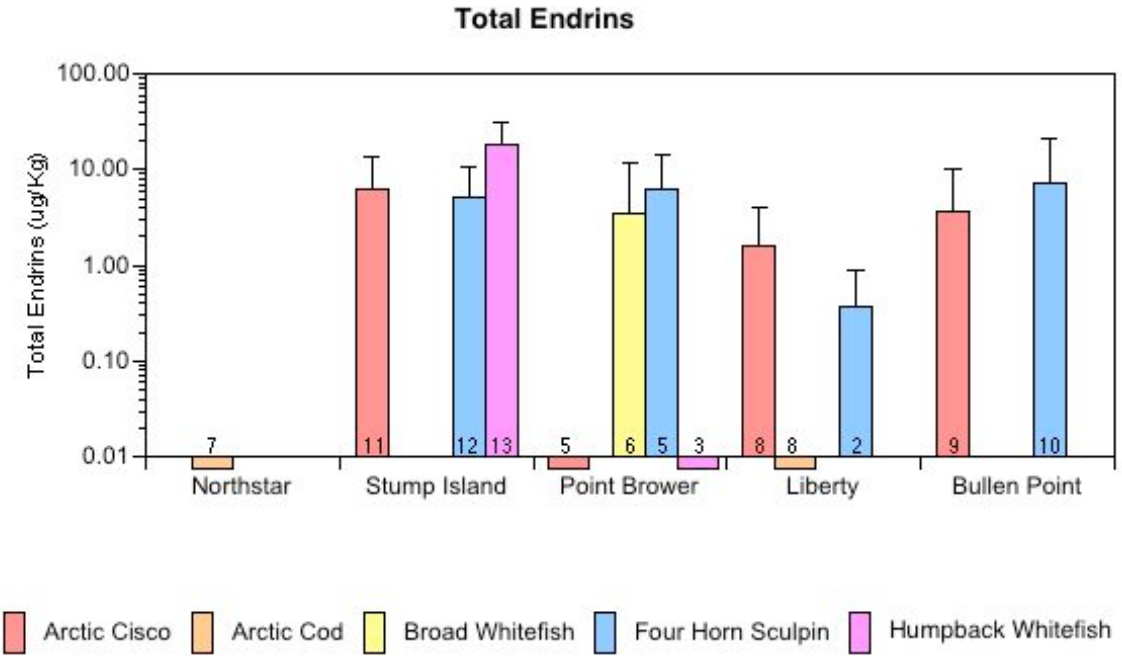

Figure 13. Total endrins (lipid normalized wet-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar. Values below the x-axis are between 0 and the lowest value on the y-axis.

3.3 P4501A In Fish Tissues

Only hepatocytes and gut mucous epithelia cells showed much induction in the species we captured, and even here the induction was low to moderate. We present the data for these two cell types in Table 10, Figures 14 and 15. Arctic Cod and Four Horn Sculpin both showed significant variation due to site ($p=0.0141$ and $P=0.0090$, respectively) for P4501A abundance in liver hepatocytes. For Arctic Cod, Northstar fish had significantly greater P4501A abundance than did specimens collected from the Liberty site—these were the only two sites where they were collected. For Four Horn Sculpin, Point Brower and Stump Island had comparable values, which were significantly higher than fish from Bullen Point. The remainder of the fish species showed no difference in liver hepatocyte P4501A abundance. There was not a significant effect of site on P4501A abundance in gut epithelial cells.

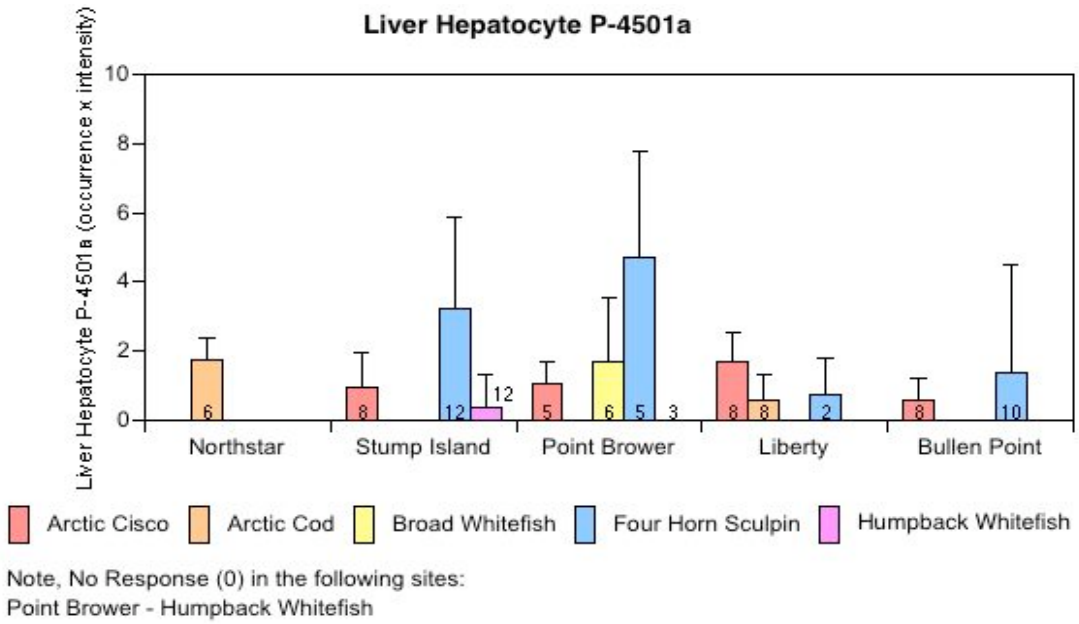

Figure 14. Liver hepatocyte P4501A staining scores by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar. The value for Humpback Whitefish at Point Brower was zero and is represented only by the number of fish (3).

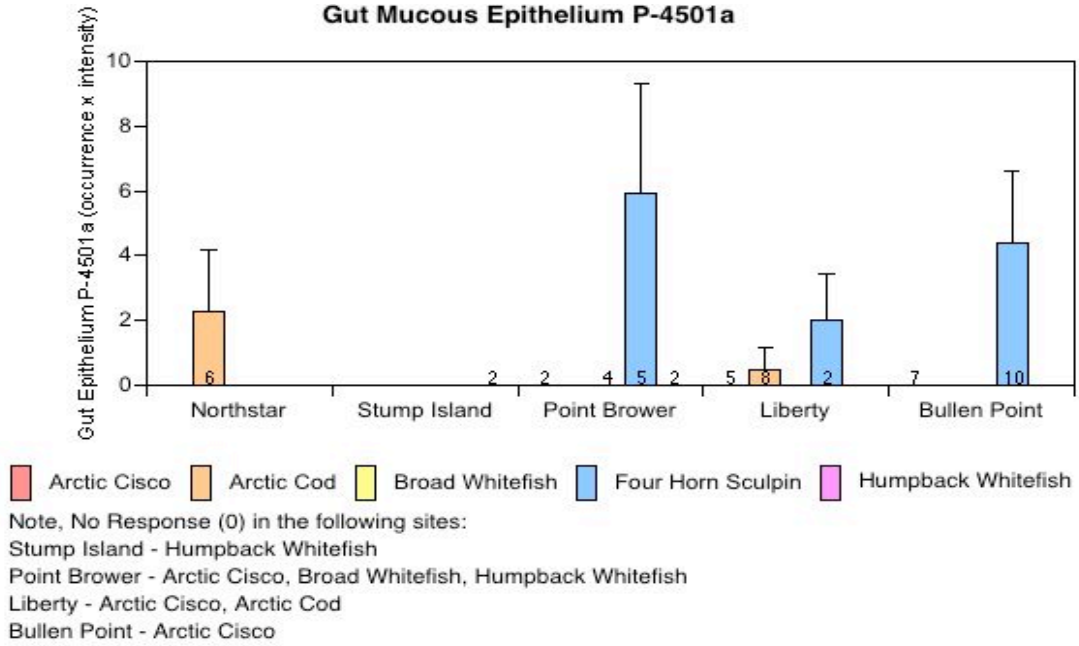

Figure 15. Gut mucous epithelium P4501A staining scores by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar. Values that averaged zero are represented on the x-axis by a number indicating the number of fish.

3.3.1 Relationship of Whole-Body Concentrations of Organic Chemicals to P4501A

Stepwise multiple regression for each fish species using the P4501A scores for liver hepatocytes and gut mucus epithelium and low-molecular-weight PAHs, high-molecular-weight PAHs and PCB whole-body concentrations indicated significant relationships in two species, Arctic Cod and Four Horn Sculpin. In the Arctic Cod, PCB concentrations were related to the abundance of P4501A in hepatocytes. In the Four Horn Sculpin, low-molecular-weight PAHs were positively related to P4501A abundance in gut epithelia (Table 10).

Table 10. Significant results of backward stepwise multiple regressions to determine the effects of lipid normalized contaminant concentrations (i.e., sum of PCBs, sum of low-molecular-weight PAHs, sum of high-molecular-weight PAHs, sum of pesticides) on P4501A activity for two tissues in five species of fish. All values were log transformed (log_{10} for contaminants, log_{10}+1 for P4501A).

Tissue/Cell and Species	r^2	p	Regression Equation
Liver Hepatocyte P4501A			
Arctic Cod	0.6042	**0.0011**	Y = 0.3876 PCBs - 0.6028
Gut Mucus Epithelium P4501A			
Four Horn Sculpin	0.2193	**0.0580**	Y = 0.3833 low-molecular-weight PAHs – 0.3770

3.4 Bile Hydrocarbons

Bile hydrocarbons and hydrocarbon metabolites were determined for two PAHs, phenanthrene and benzo(a)pyrene, which represent compounds with three and four aromatic rings, respectively. These also respectively represent our low-molecular-weight and high-molecular-weight PAH compound classes. In general phenanthrene is more representative of fresh petroleum and benzo(a)pyrene weathered petroleum and, especially, pyrogenic sources (e.g., forest fires, internal combustion engines). All species with bile samples were tested for site differences and three cases of significant variation were found. There was a highly significant variation due to site for phenanthrene equivalents in the bile of Four Horn Sculpin, in which comparable concentrations were found at Bullen Point, Stump Island and Liberty, which were all higher than Point Brower (Table 9, Figures 16 and 17). One individual Arctic Cisco at Bullen Point had a value of 10,000 ng/g phenanthrene equivalents. Benzo(a)pyrene equivalents exhibited highly significant variation due to site in Arctic Cisco, for which Bullen Point was higher than Stump Island and Point Brower. In Four Horn Sculpin, benzo(a)pyrene equivalents were significantly higher at Stump Island than at Point Brower. Many of the fish assayed had values in excess of 5,000ng/g B(a)P equivalents.

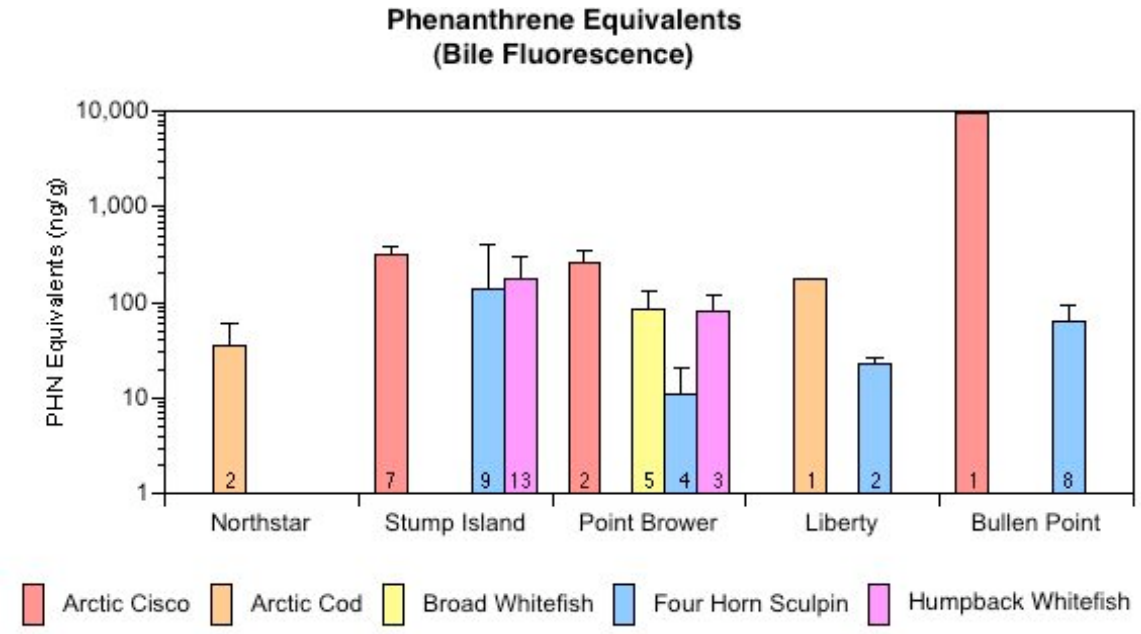

Figure 16. Phenathrene equivalents by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

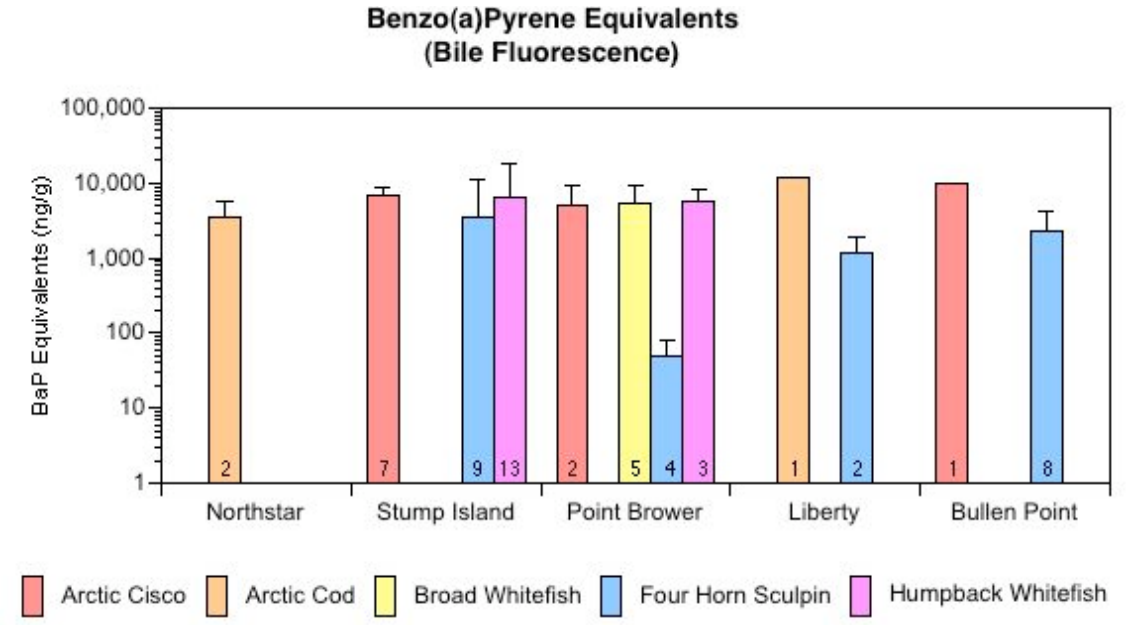

Figure 17. Benzo(a)pyrene equivalents by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

3.5 Metals in Fish

Tissue aliquots were analyzed from all species for 12 metals. The data were generally analyzed in the same way that those for organic compounds were. Using two-way analysis of variance with site and size of fish as the dependent variables and whole-body-metal concentration as the independent variable. The results of these statistical analyses are presented in Table 11.

Table 11. Results of one-way ANOVA for the effects of site, with weight as a covariate, on the concentrations of trace metals in five species of fish. Statistically significant results ($p \leq 0.05$) are indicated by bold type.

		Weight	Site		Interaction
Analyte Group & Species	r^2	p	p	Student's[a,b]	p
Arsenic					
Arctic Cisco	0.3756	**0.0041**	0.0949	SI=L=BP=PB	0.9550
Arctic Cod	**0.0041**	0.8974	0.8433	L=NS	0.3472
Broad Whitefish	0.5788	0.0790	-	PB	-
Four Horn Sculpin	0.6531	**0.0062**	**0.0008**	SI=L=PB>BP	0.2503
Humpback Whitefish	**0.0337**	0.7424	0.5156	SI=PB	**0.0177**
Barium					
Arctic Cisco	0.2359	**0.0492**	0.3593	SI=PB=BP=L	0.9664
Arctic Cod	0.3013	0.2949	0.1870	NS=L	0.5971
Broad Whitefish	0.5125	0.1096	-	PB	-
Four Horn Sculpin	0.4055	0.6967	**0.0053**	PB=L>BP, PB>SI, L=SI, SI=BP	0.6778
Humpback Whitefish	0.1263	0.3246	0.2019	SI=PB	0.6144
Cadmium					
Arctic Cisco	0.2954	0.1671	**0.0485**	PB=L=SI, PB>BP, L=SI=BP	**0.0252**
Arctic Cod	0.3135	0.9374	0.0611	NS=L	0.5804
Broad Whitefish	0.1443	0.4133	-	PB	-
Four Horn Sculpin	0.4885	**0.0487**	0.0601	SI=L=BP=PB	0.9280
Humpback Whitefish	0.3982	0.3055	**0.0143**	SI>PB	0.9451
Chromium					
Arctic Cisco	0.3813	0.7748	**0.0040**	SI=L>BP, SI>PB=BP, L=PB	0.4336
Arctic Cod	0.0771	0.7150	0.4940	NS=L	0.2898
Broad Whitefish	0.3758	0.1957	-	PB	-
Four Horn Sculpin	0.3469	0.8765	**0.0289**	L=PB>SI, PB=BP, BP=SI	0.8805
Humpback Whitefish	**0.0293**	0.6453	0.9957	PB=SI	0.8825
Copper					
Arctic Cisco	0.1660	0.1926	0.4451	SI=L=BP=PB	0.7059
Arctic Cod	0.5481	**0.0039**	0.4051	NS=L	0.6182
Broad Whitefish	0.5719	0.0819	-	PB	-
Four Horn Sculpin	0.2265	**0.0243**	0.5825	L=SI=PB=BP	0.2107
Humpback Whitefish	0.3477	0.6678	**0.0468**	SI>PB	0.8456

		Weight	Site		Interaction
Analyte Group & Species	r^2	p	p	Student's[a,b]	p
Iron					
Arctic Cisco	0.2936	**0.0319**	0.1328	BP=SI=L=PB	0.7236
Arctic Cod	0.5376	0.8356	**0.0071**	NS>L	0.9307
Broad Whitefish	0.5797	0.0786	-	PB	-
Four Horn Sculpin	0.2550	0.4266	0.0654	L=PB=SI=BP	0.6798
Humpback Whitefish	0.1175	0.2304	0.4486	SI=PB	0.8735
Lead					
Arctic Cisco	0.1298	0.5195	0.3540	L=SI=BP=PB	0.8177
Arctic Cod	0.3116	0.9453	0.0617	NS=L	0.1033
Broad Whitefish	0.2836	0.2767	-	PB	-
Four Horn Sculpin	0.1627	0.3521	0.2294	BP=PB=L=SI	0.0530
Humpback Whitefish	0.4875	0.0766	**0.0056**	SI>PB	0.3129
Mercury					
Arctic Cisco	0.5111	0.8321	**0.0002**	PB>SI>L=BP	0.4379
Arctic Cod	0.4009	0.4107	0.0552	NS=L	0.2757
Broad Whitefish	0.2639	0.2973	-	PB	-
Four Horn Sculpin	0.4188	**0.0092**	0.4984	SI=BP=PB=L	**0.0153**
Humpback Whitefish	0.5096	0.1726	0.0896	SI=PB	0.4997
Nickel					
Arctic Cisco	0.2001	0.6212	0.1039	SI=L=BP=PB	0.1535
Arctic Cod	0.0847	0.5739	0.5696	NS=L	0.4935
Broad Whitefish	0.1807	0.4008	-	PB	-
Four Horn Sculpin	0.0719	0.8853	0.6533	L=SI=PB=BP	0.5701
Humpback Whitefish	0.1415	0.1850	0.2578	SI=PB	0.7548
Selenium					
Arctic Cisco	0.1898	0.2688	0.2055	PB=L=SI=BP	0.7670
Arctic Cod	0.6337	0.9151	**0.0016**	L>NS	0.3972
Broad Whitefish	**0.0185**	0.7974	-	PB	-
Four Horn Sculpin	0.0982	0.3870	0.4797	PB=BP=L=SI	0.9731
Humpback Whitefish	0.2642	0.0573	0.1120	SI=PB	0.1486
Vanadium					
Arctic Cisco	0.2762	**0.0396**	0.1869	PB=L=SI=BP	0.9545
Arctic Cod	0.3229	0.3441	**0.0438**	L>NS	0.3846
Broad Whitefish	**0.0015**	0.9424	**-**	PB	-
Four Horn Sculpin	0.0877	0.5840	0.5327	L=PB=SI=BP	0.4450
Humpback Whitefish	0.1256	0.6248	0.2105	SI=PB	0.9890
Zinc					
Arctic Cisco	0.4020	0.4332	**0.0068**	PB=SI=L>BP	0.9730
Arctic Cod	0.3326	**0.0391**	0.4367	L=NS	0.8970
Broad Whitefish	0.0578	0.6463	-	PB	-
Four Horn Sculpin	0.1161	0.9008	0.3906	L=PB=SI=BP	0.6046
Humpback Whitefish	0.5310	0.1118	**0.0035**	SI>PB	0.7391

[a] Student's *a posteriori* results are for Student's t test for least significant means. Values are arranged with the highest means on the left and the lowest on the right.
[b] BP = Bullen Point, L = Liberty, NS = Northstar, PB = Point Brower, SI = Stump Island.

Arsenic - Four Horn Sculpin was the only species that had significant variability in As concentration due to site. Stump Island, Liberty and Point Brower had comparable concentrations, which were higher than Bullen Point. There was only one significant interaction term; that between site and weight for Humpback Whitefish. Mean As concentrations ranged between about 2 and 14 $\mu g/g$ (dry-weight) for the five species analyzed from the sites, with Arctic Cod averaging above 10 $\mu g/g$ (dry-weight) and Humpback Whitefish below 5 $\mu g/g$ (dry-weight) (Table 12, Figure 18).

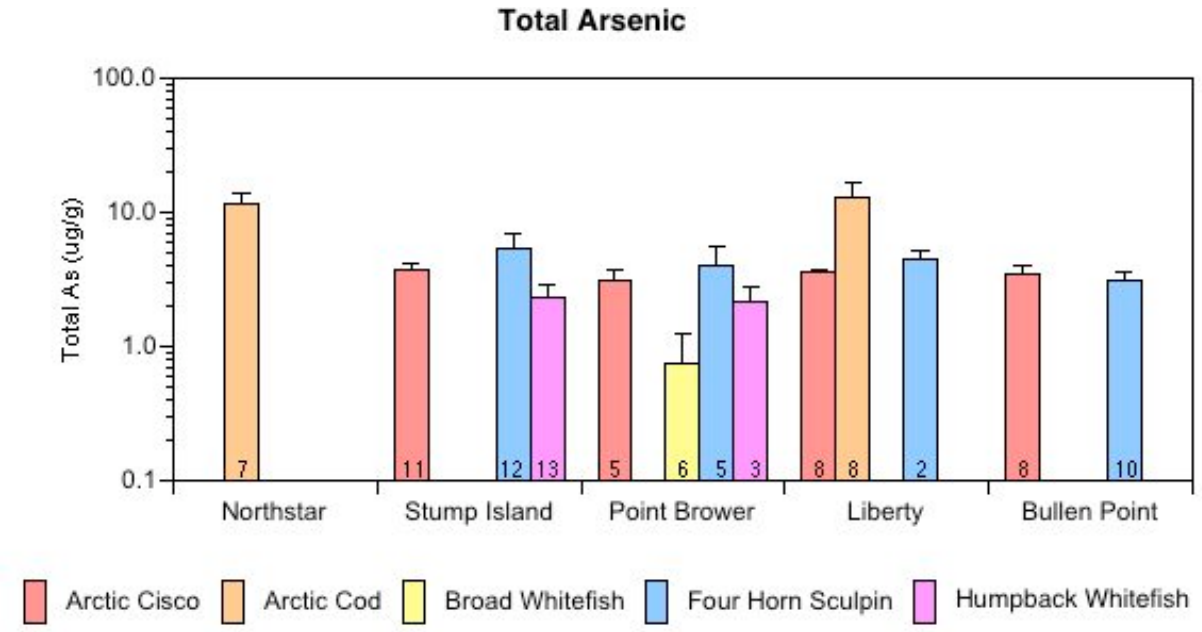

Figure 18. Total arsenic (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Barium - Only Four Horn Sculpin displayed significant variability in Ba concentrations with site ($p=0.0053$). Point Brower and Liberty had equivalent concentrations and they were higher than Bullen Point. No other species showed significant variation in Ba concentrations with site (Table 12, Figure 19).

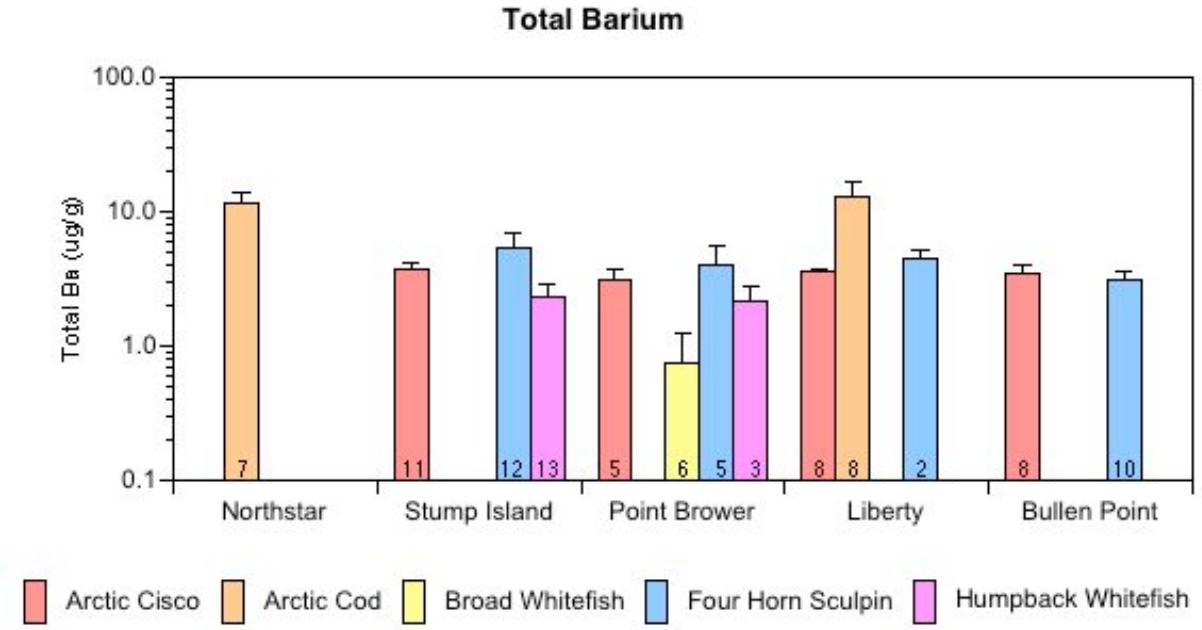

Figure 19. Total barium (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Cadmium - This is an interesting element because of its dramatic change in bioavailability between fresh and salt water and the subsequent effect on toxicity (Hall and Anderson, 1995). Cadmium is complexed by chloride in seawater and not as nearly as available as in freshwater. Two species showed significant differences due to site, Arctic Cisco and Humpback Whitefish. Arctic Cisco also showed a site-weight interaction. Arctic Cisco at Point Brower had higher concentrations than those at Bullen Point, consistent with and possibly due to higher availability of cadmium in the freshwater outflow of the Saganvirtok River (Table 12, Figure 20). Humpback Whitefish also had significantly higher concentrations at Stump Island than at Point Brower ($p=0.01$).

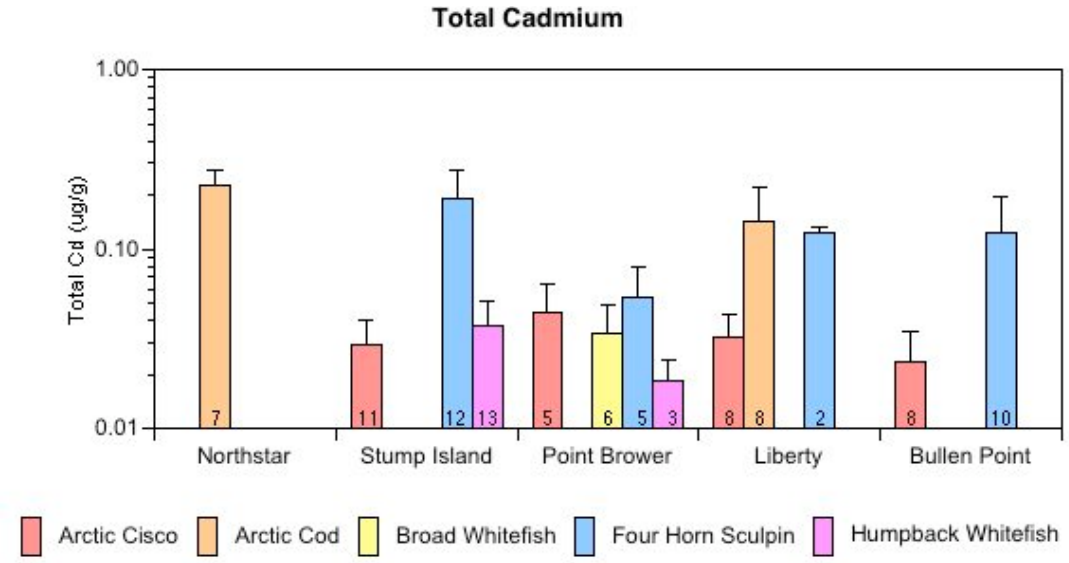

Figure 20. Total cadmium (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Chromium - Chromium concentrations varied with site for the Arctic Cisco and Four Horn Sculpin (Table 12, Figure 21). For Arctic Cisco, Stump Island and Liberty had higher concentrations than did Bullen Point. For Four Horn Sculpin, Liberty and Point Brower had higher concentrations than did Stump Island.

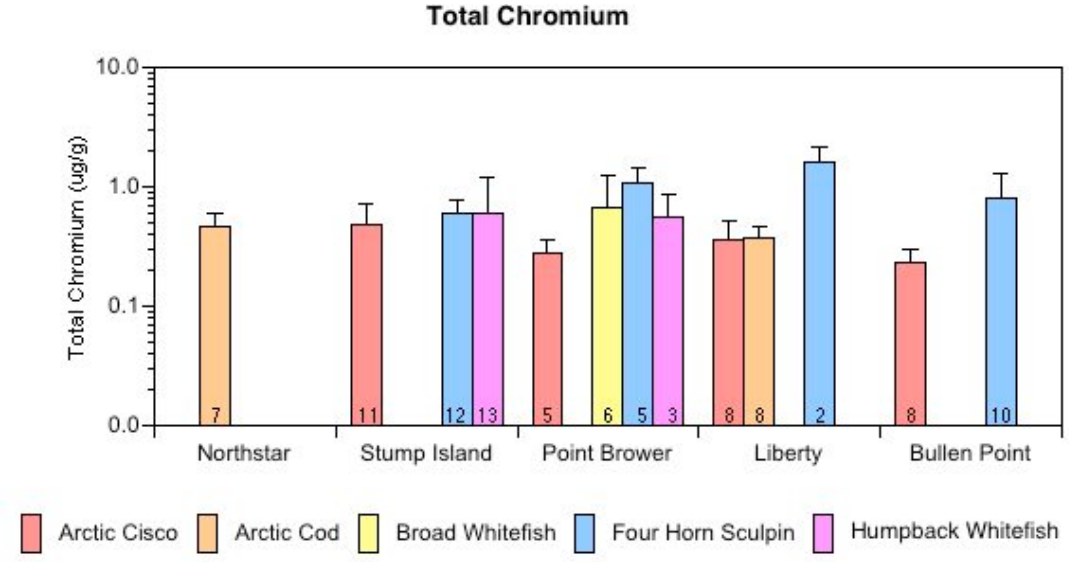

Figure 21. Total chromium (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Copper - Whole-body concentrations of copper ranged from about 3 to 20 ppm. There was one significant site difference, for Humpback Whitefish, in which Stump Island was higher than Point Brower (Table 12, Figure 22).

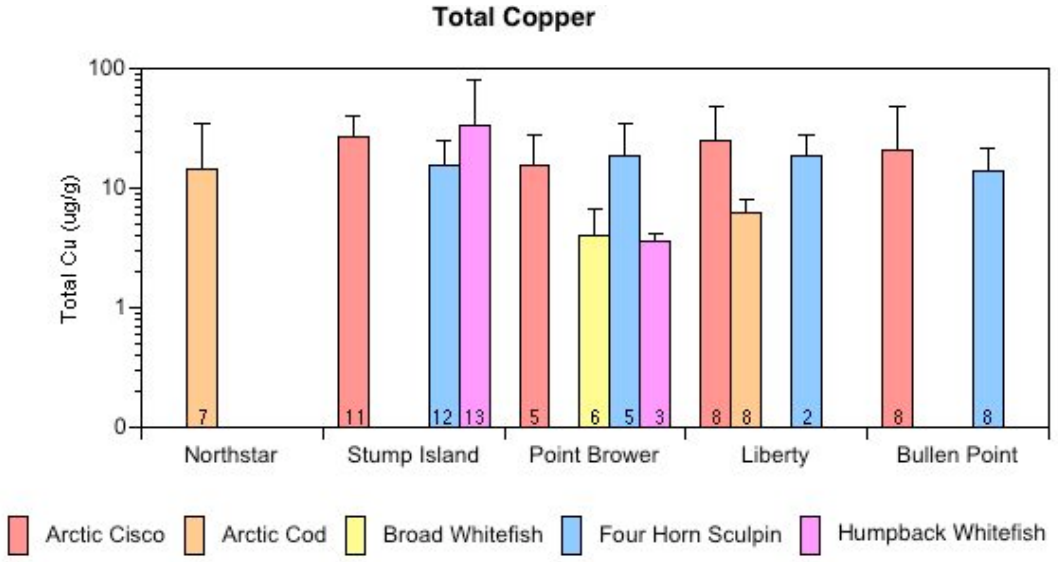

Figure 22. Total copper (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Iron - Only the Arctic Cod had significant differences due to site, with Northstar having higher concentrations than Liberty. Four Horn Sculpin also had higher concentrations of Fe compared to the other species with the highest mean concentrations at Liberty. However, the high variability in Fe concentrations in this species precluded finding any significant site differences (Table 12, Figure 23).

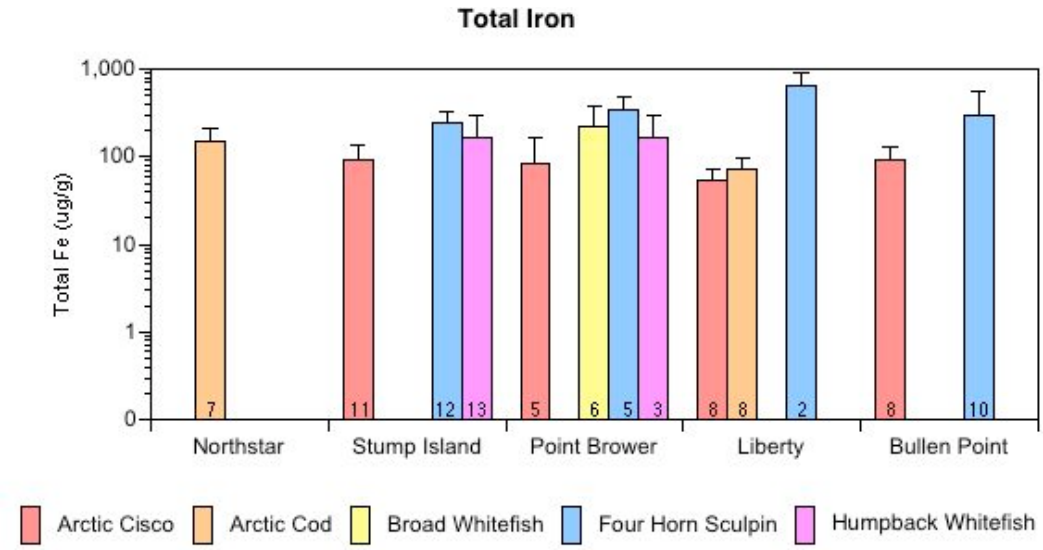

Figure 23. Total iron (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Lead - There was one significant variation in Pb concentrations due to site, for Humpback Whitefish. (Table 12, Figure 24). For this species, Stump Island was higher than Point Brower.

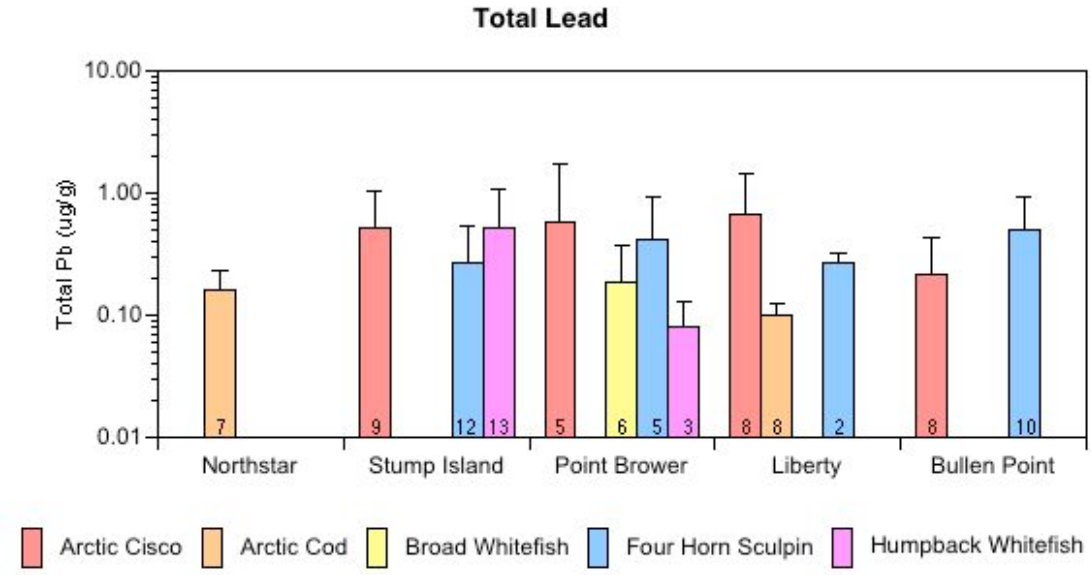

Figure 24. Total lead (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Mercury - In Arctic Cisco, mercury concentrations varied significantly with site. In this species, Point Brower fish had significantly greater mercury concentrations than did any other sites, and Stump Island had higher concentrations than either Liberty or Bullen Point. When Four Horn Sculpins occurred with other species in the analyzed collections, they had the highest concentrations of mercury. In Four Horn Sculpin there was also a significant interaction between site and weight (Table 12, Figure 25).

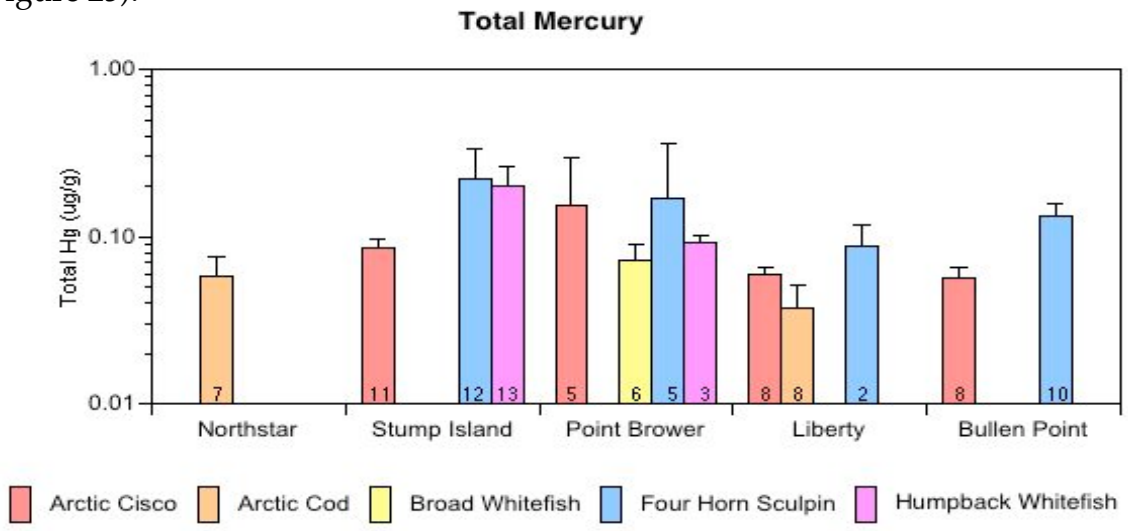

Figure 25. Total mercury (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Nickel - There were no significant effects of site on Ni whole-body concentrations in any of the fish species. There were also no significant interactions of site with fish weight (Table 12, Figure 26).

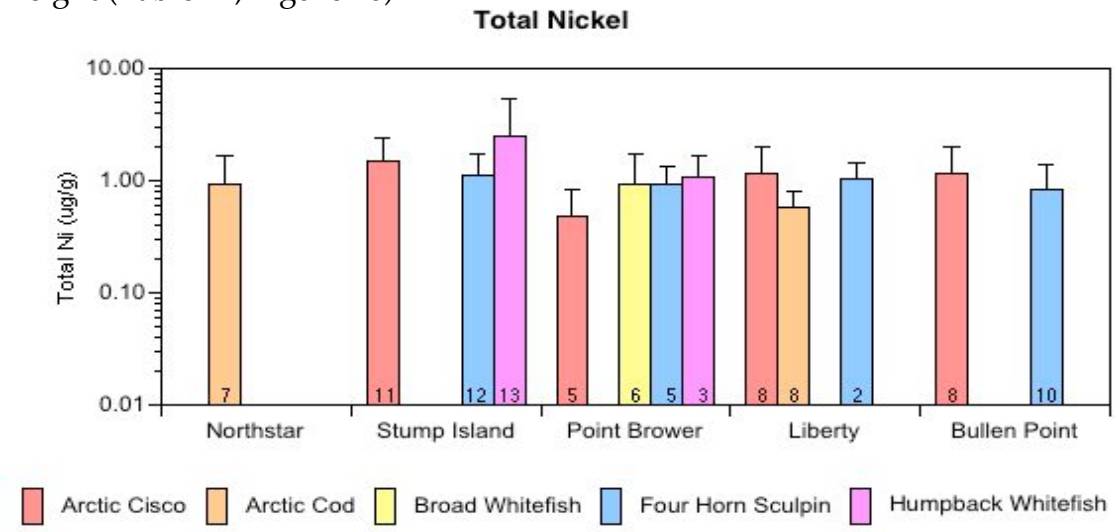

Figure 26. Total nickel (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Selenium - There was one species that had differences in Se concentration due to site, Arctic Cod. Arctic Cod from Liberty had significantly higher concentrations than did those from Northstar (Table 12, Figure 27). In three out of four sites where Four Horn Sculpin occurred with other species, they had the highest mean concentrations.

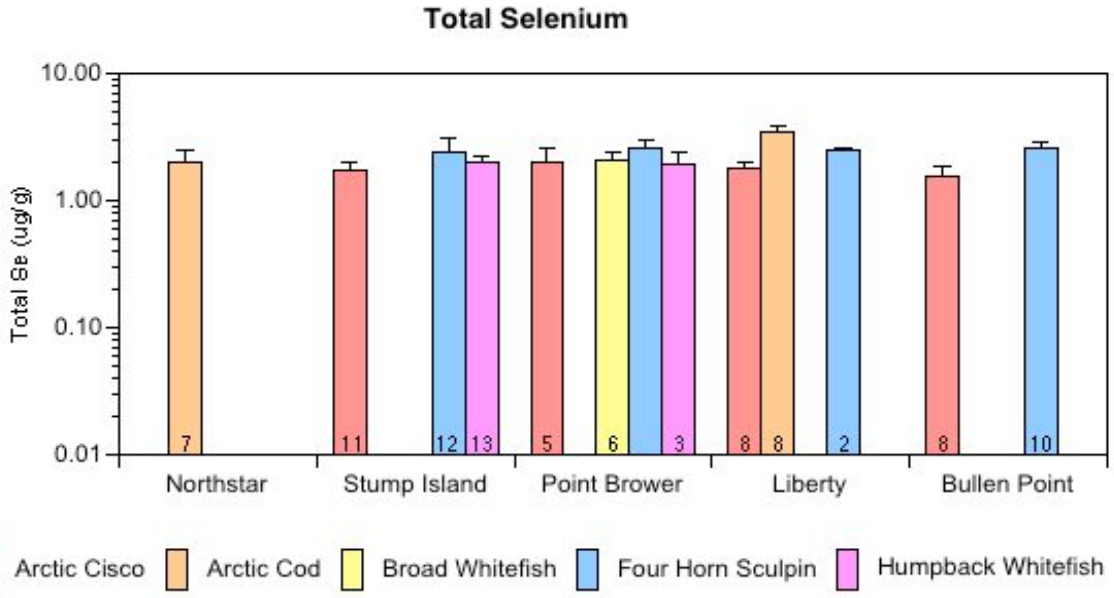

Figure 27. Total selenium (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Vanadium - There was only one species in which the concentration of V varied significantly with site, the Arctic Cod. As for selenium, the Liberty fish had significantly higher concentrations than did the Northstar fish (Table 12, Figure 28). In three out of four sites where Four Horn Sculpin occurred with other species, they had the highest mean concentrations.

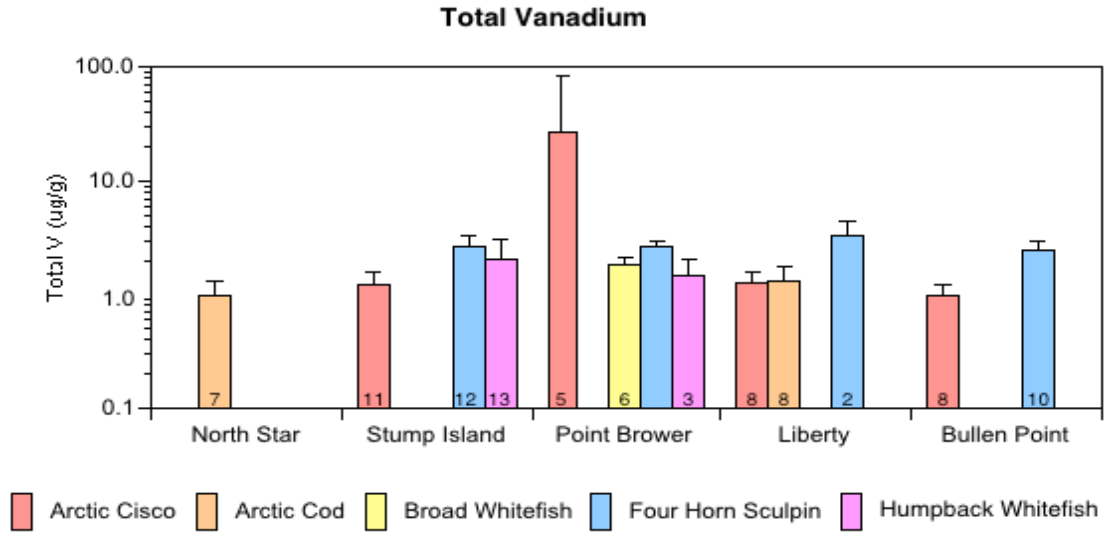

Figure 28. Total vanadium (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

Zinc - The Arctic Cisco and Humpback Whitefish were the only species in which there was significant variability in whole-body Zn concentrations due to site. For Arctic Cisco, Point Brower, Stump Island and Liberty were equivalent, but they were all higher than Bullen Point. For Humpback Whitefish, Stump Island fish had higher concentrations than Point Brower fish (Table 12, Figure 29). In three out of four sites where Four Horn Sculpin occurred with other species they had the highest mean concentrations.

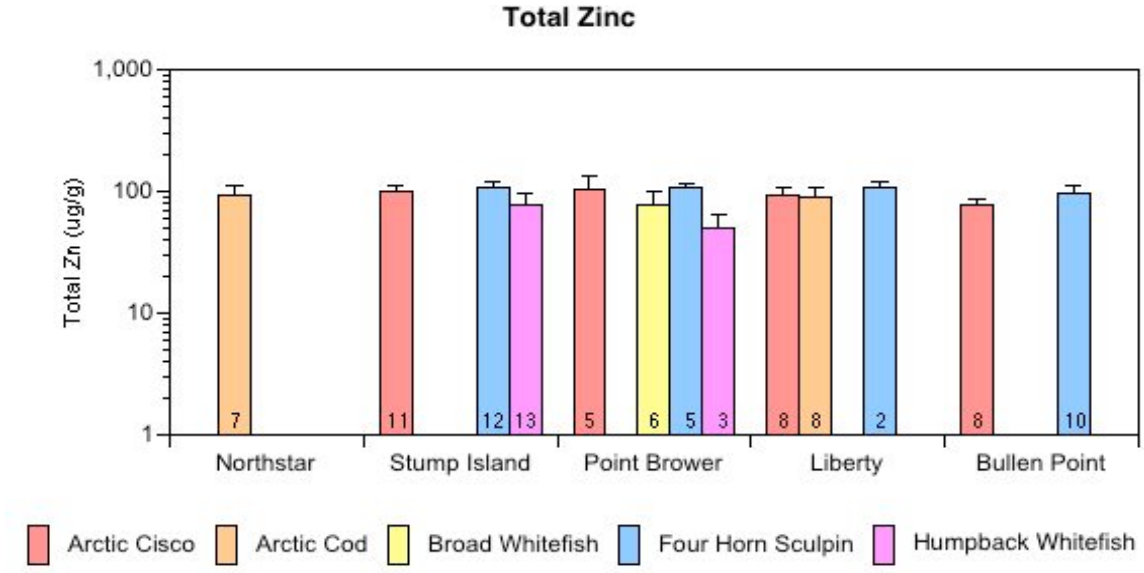

Figure 29. Total Zinc (dry-weight) concentrations by species and site. Error bars depict one standard deviation and the numbers of individual fish making up the samples are indicated at the bottom of each bar.

3.6 Cluster Analyses

In an attempt to further understand the patterns of variability of contaminants in the various species of fish, we carried out several kinds of cluster analyses (Figures 30, 31, 32 and 33). The first cluster analysis was based on standardized mean concentrations of all organic analytes. In the results of that analysis there were three separate groups found to be significant at the highest level of separation: 1) Four Horn Sculpin from Point Brower and Stump Island (green), 2) Four Horn Sculpin from Bullen Point (blue), and 3) All remaining site-species combinations (red). While there was one site-species interaction for Four Horn Sculpin in the third cluster, this result generally suggests that Four Horn Sculpin show innate patterns of variability that are more similar within species than with other species in this study.

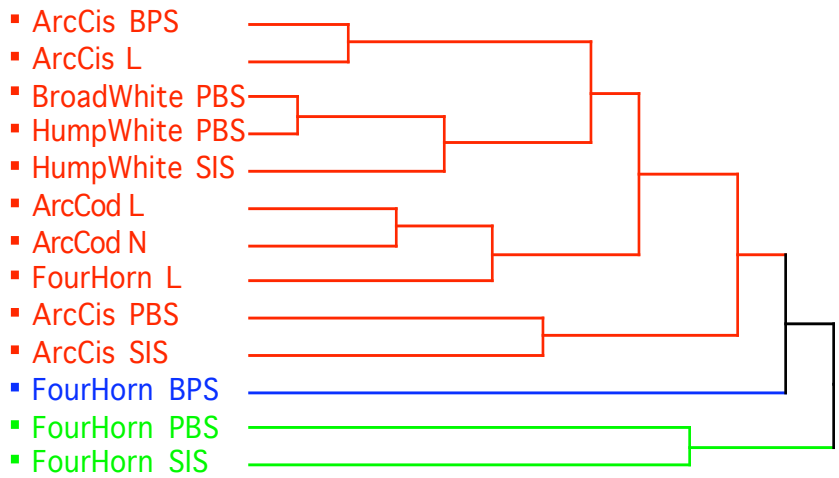

Figure 30. Clusters of species/site combinations based on standardized mean concentrations of all organic analytes. Colors indicate separate clusters.

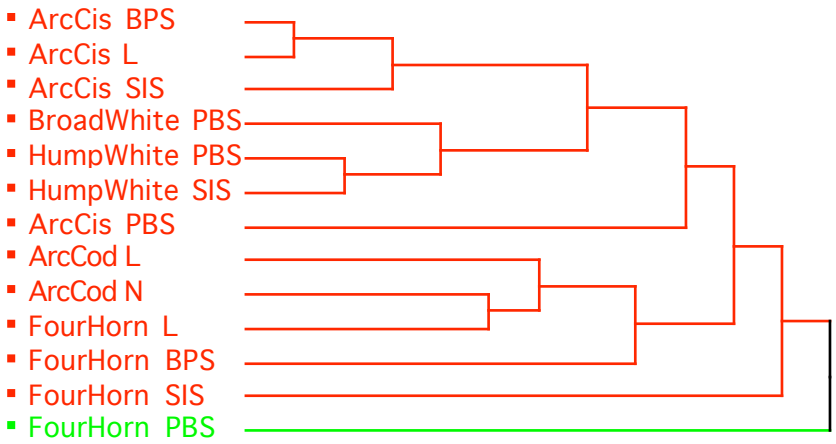

Figure 31. Clusters of species/site combinations based on standardized mean concentrations of PAH analytes. Colors indicate separate clusters.

In the second cluster analysis we did a similar procedure, but using only all of the PAHs. The results of this analysis were similar to those for all organic analytes in that the Four Horn Sculpin from Bullen Point clustered separately from all the other site-species combinations (Figure 31). A similar pattern emerged for the PCB congeners, the Bullen Point Four Horn Sculpin stood out as different from the other site-species combinations (Figure 32). The analysis of pesticides only produced two clusters of about equal size in which no particular species or sites stood out as dominating either cluster (Figure 33).

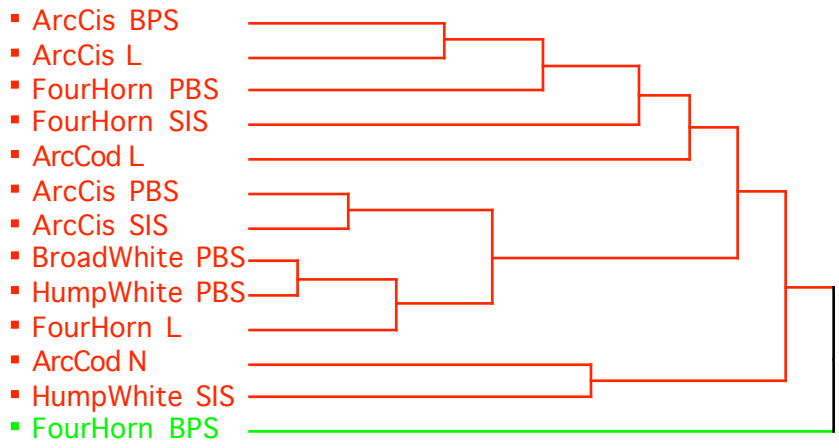

Figure 32. Clusters of species/site combinations based on standardized mean concentrations of PCB congeners. Colors denote separate clusters.

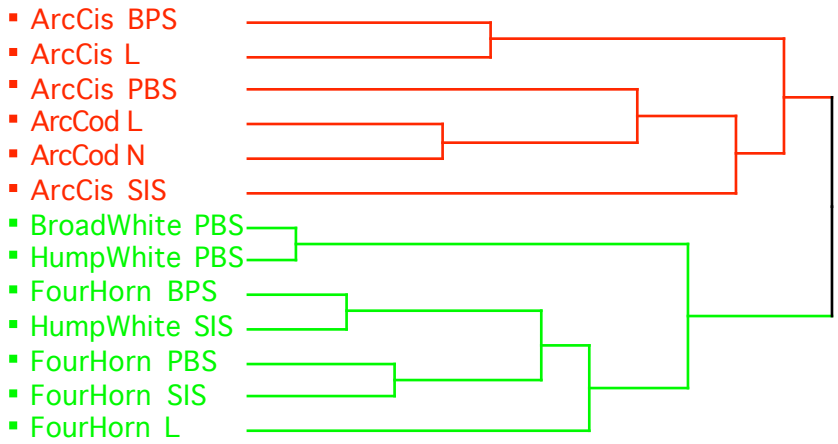

Figure 33. Clusters of species/site combinations based on standardized mean concentrations of pesticide analytes. Colors denote separate clusters.

4.0 Discussion

The objective of Task 8 of the ANIMIDA project was to determine if trace substances in the North Slope oil field development areas might be resulting in uptake of trace substances in fish to concentrations in excess of surrounding natural conditions. There are some general considerations in correctly interpreting the data presented in the results section with regard to this objective.

First, for substances that occur naturally, i.e., trace metals and PAHs, some means of partitioning what might be natural and what might be anthropogenic, and within the anthropogenic category what is due to oil field operations, is necessary to reach project goals. This can be done in a two ways:

1. Comparison of tissue concentrations in areas of human activity with sites representing background conditions. Elevated concentrations in tissues can result from local anthropogenic sources. There have been both military and oil field development activities in the study area and these influences should be considered. For example, in our fieldwork at the Bullen Point site we saw a petroleum-like material seeping into the ocean at the Distant Early Warning (DEW) military installation. There are also potential natural sources and natural conditions that might contribute more biologically available components in certain areas. For example, freshwater from the Sagavanirktok River might change speciation for naturally available cadmium and make it more likely to accumulate in fish near the River delta (There was some suggestion of this in the data for Arctic Cisco but several sites were equally high). Differential loading of organic mater into sediments might also change rates of Hg methylation, and, hence, increase Hg availability to fish locally. There also local sources for PAH that might include seepage and natural peats (Valette-Silver, 1999).

2. Use of diagnostic ratios between elements or compounds that might indicate a source of the trace substances in the tissues. This works well for sediments, but the approach has to be used cautiously with organisms which might differentially take up, metabolize or excrete compounds, distorting the ratios over the source ratios. Copper and zinc, for instance, are regulated physiologically in fish. It appears that for organic compounds many invertebrates are much more useful than are vertebrates because fish, birds and mammals metabolize many organic compounds at appreciably greater rates than invertebrates.

One other important process to consider is metabolism of trace organic compounds. The P4501A biomarker is an enzyme that is both induced by some organic contaminants, e.g., some PCBs and PAHs, and that can metabolize a variety these and other compounds as well. So, interpreting the data on PAH in tissues should take this into account. It is possible to have little detectable PAH in an animal with an active P4501A enzyme system when it is being exposed to relatively high and potentially harmful concentrations in the environment. In fact, it has been experimentally demonstrated that long-term exposure of fish to petroleum-contaminated sediments can result in no detectable PAH in tissue after 60 days, while the fish is still being exposed (McCain et al., 1978). So tissue concentrations of PAH in

fish may not be a reliable indicator of what is being absorbed, quickly metabolized and excreted. It is for this reason that the enzyme biomarker P4501A was measured in this study in addition to the PAH concentrations in the tissues. This enzyme is induced within several hours of significant exposure to oil and PAH and may persist on the order of weeks after exposure (Elskus and Stegeman, 1989; Stegeman and Kloepfer-Sams, 1989; Spies et al., 1982). This enzyme can also be induced by PCBs, but since the PCBs appeared to be generally uniformly distributed in fish at different stations in this study, its contribution to enzyme induction is likely to be uniform between stations. The second biomarker used in this study is the concentration of PAH metabolites in the bile. If a fish is being exposed to PAH and they are being taken up from food, sediment or water, and being metabolized, they will be excreted in the bile (Krahn et al, 1984). This biomarker is thought to reflect mainly the bioavailable aromatic hydrocarbons in the last few meals eaten by the fish.

An important consideration in data interpretation is the number of fish analyzed at each site. Our data is such that the power of any conclusions that are drawn is low. For example, most contrasts between Liberty and Northstar are based on 7 or 8 individual fish at each site, whereas similar work done elsewhere would be based on 15-20 or more individuals at a site. In general, trace constituent concentrations follow log normal distributions in exposed populations and this sort of distribution requires larger numbers of samples to capture variability than if the distribution was a normal one.

Also, we have reported tissue residue concentrations of several trace substances where site is a significant source of variation, but for which we have no immediate explanation, neither natural or anthropogenic enrichment. It should be kept in mind that with marginal numbers of samples for many analyte-species combinations that some of these differences between sites may be due to chance. In addition, with a large number of analyses one might expect chance occurrences of significant relationships. There were 62 ANOVAs (not counting the total PAHs) that were carried out for combinations of species and trace organic substances and biomarkers and 60 ANOVAs for the metals for a total of 122 ANOVAs. At an alpha level of 0.05, as was used in this study, it would be expected that about 6 of the ANOVAs would return alpha values of 0.05 or less just on the basis of chance. There were 16 ANOVA results for organic compounds/ biomarkers with alpha values of 0.05 or less and 14 for metals with alpha values of 0.05 or less. So out of the 30 trace-substances/species combinations that returned alpha values of 0.05 or less, about 10% could have been due to chance. We could have been more conservative in our approach by reducing the conventional alpha level in the ANOVAs by dividing by the number of ANOVAs that were carried out. However, we felt that this approach is too restrictive in an exploratory study and would rather have some unexplained results than miss real effects.

In Table 12, we present the range of mean concentrations for the fish analyzed from the collection stations. So although there are low numbers of fish from individual stations these mean ranges provide a very good picture of the trace substance concentrations in the development area of the Beaufort Sea.

Table 12. Ranges of Mean Concentration Values at Collection Sites.

Means (Range)															
Species	Total PAH	Total PCBs	Total Pests	As	Ba	Cd	Cr	Fe	Cu	Hg	Ni	Pb	Se	V	Zn
Arctic Cisco	232.9-464.5	68.2-83.4	166.2-346.3	3.1-3.7	3.07-73.64	0.02-0.04	0.23-0.49	53.5-90.7	15.4-21.2	0.057-0.154	0.476-1.144	0.213-0.675	1.55-2.00	0.97-26.9	79.0-105.4
Arctic Cod	599.7-797.1	234.3-331.9	208.7-224.5	11.6-13.1	11.6-13.1	0.14-0.23	0.37-0.46	72.2-151.9	6.17-14.36	0.038-0.058	0.58-0.93	0.10-0.16	1.98-3.4	0.97-1.28	91.4-93.5
Broad Whitefish	*230.3	52.3	81.4	0.8	0.762	0.034	0.68	226.0	4.0	0.073	0.942	0.187	2.053	1.855	78.517
Four Horn Sculpin	610.2-1654.3	100.8-2310	162.9-222.0	3.06-5.5	3.05-5.45	0.054-0.189	0.80-1.61	238.8-663.0	14.1-18.9	0.09-0.22	0.83-1.11	0.26-0.50	2.38-2.63	2.49-3.29	96.7-106.3
Humpback Whitefish	105.1-137.0	118.8-610.6	125.9-204.1	2.2-2.4	2.157-2.353	0.018-0.038	0.56-0.61	166.9-168.6	3.7-33.0	0.092-0.198	1.097-2.462	0.08-0.5242	1.92-2.00	1.47-2.10	50.40-78.37

*Broad Whitefish were collected only at one site, Point Brower.

Finally, we present two ways of looking at the current sampling efforts and how these inform our ability to detect change over time, or differences between collections in the contaminant measures in fish undertaken in this study. First, in Figure 34, we use the variability in some of the organic contaminant classes and markers among the Four Horn Sculpin to determine how long, using annual sampling, it would take to detect different percent changes in this species. The plot shows, for example, that it would take only another year of sampling to detect a 50% change in the concentrations of total pesticides for the Four Horn Sculpin. On the other hand, it would take four additional years to detect a 50% change in low-molecular-weight PAHs, 6 years to detect a similar change in total PCBs and liver hepatocyte P4501A and 7 years to detect this change in high-molecular-weight PAHs.

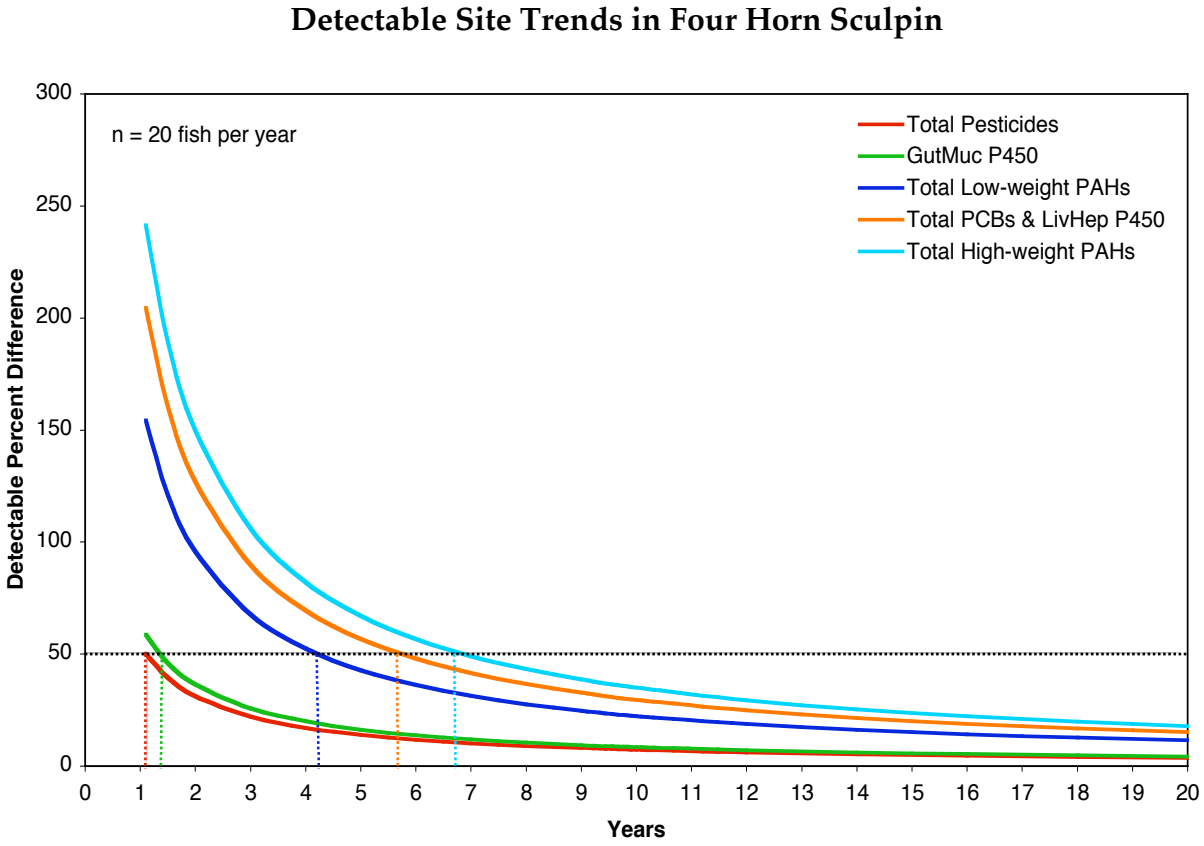

Figure 34. The number of years required to detect significant within-site trends for six variables measured in Four Horn Sculpin, if 20 fish are collected each year. The number of years required to detect significant changes of 50% are indicated for each variable. Analysis is based upon non-transformed data.

The second way of evaluating detection of differences in fish contaminant accumulation is to calculate the percent difference that can be detected in any measure between two collection areas (e.g., affected and control) as a function of the number of fish analyzed. Again, using the Four Horn Sculpin, a series of curves for a number of scenarios were generated. In Figure 35, it can be seen that for the Four Horn Sculpin the low sample numbers in this study were sufficient for detecting changes of less than 50% for most variables. However, for total high-molecular-weight PAH about 14 fish are needed to detect a true difference of 50% in means (with beta=0.80) and more than 40 fish are needed to detect a difference of 50% in liver hepatocyte P4501A.

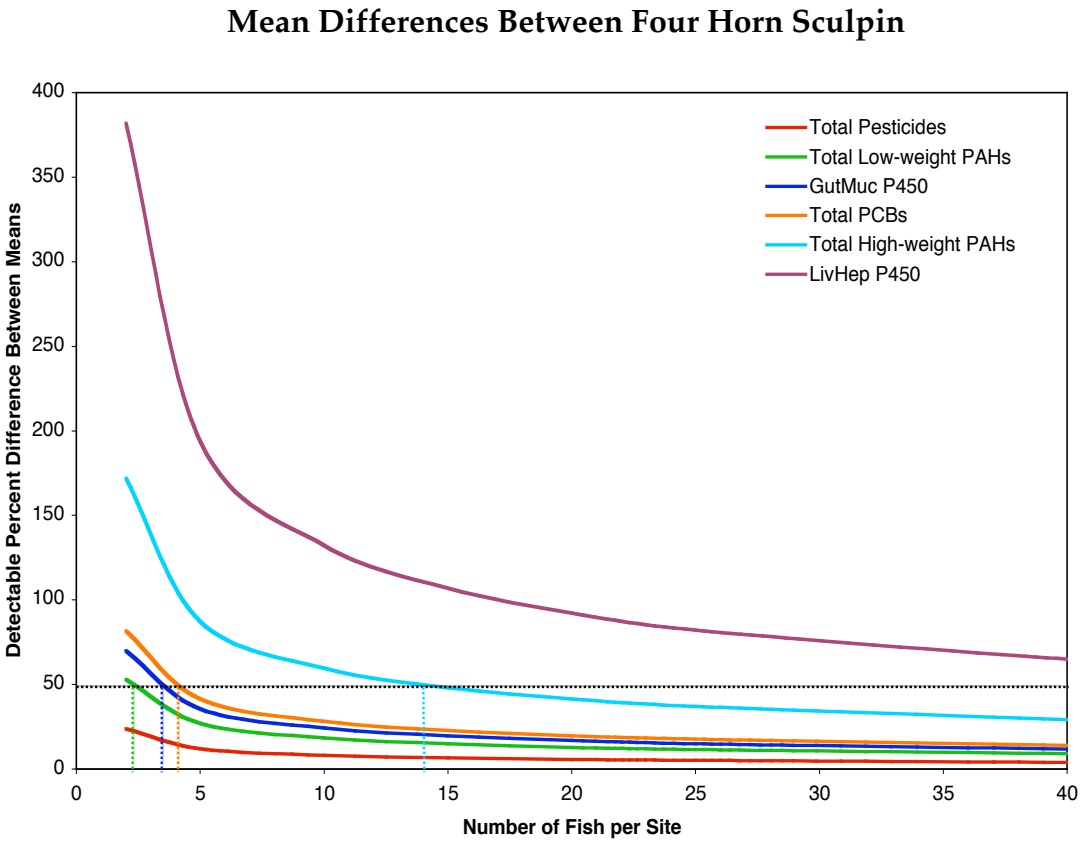

Figure 35. Relationship between numbers of Four Horn Sculpin collected from each of four sites and the percentage of difference between means that can be detected. The number of fish required to detect significant differences of 50% are indicated for each variable. These calculations are based upon log transformed data and assume that a difference with p = 0.05 would have an 80% probability of being detected.

The above discussion of power not only sets the stage for designing a future monitoring program, but also for considering the strength of any emerging trends in these 2001 data with an average about 6-7 fish per species per station.

We propose the Four Horn Sculpin as a candidate species for monitoring for a number of reasons:

1. They show a higher number of site differences than any other species except Arctic Cisco in this exploratory study (Table 13).
2. While their movements have not been explicitly studied around the project area, sculpins generally have a limited range of movement in other areas and they are likely a species that will reflect local conditions within the development area.
3. They probably represent the marine environment better than other species included in the study, especially the three anadramous species.

4. From the above analyses it is apparent that this species has relatively good power to detect change.
5. They have a rather catholic diet of epibenthic and benthic organisms and therefore probably are good integrators of contaminants derived from lower in the food web.
6. They have been recommended by the Arctic Marine Assessment Program and their use on Alaska's North Slope should contribute to a better understanding of the circumpolar distribution and fate of trace substances in the Arctic.

Table 13. Summary of significant differences for species and contaminants with respect to the effect of site.

	Four Horn Sculpin	Arctic Cod	Arctic Cisco	Humpback Whitefish	Broad Whitefish
High-molecular-weight PAH	x		x		
PCBs			x		
∑DDT			x		
Chlordanes	x		x	x	
HCH		x	x		
Endosulfans			x		
Endrins				x	
Arsenic	x				
Barium	x				
Cadmium			x	x	
Chromium	x		x		
Copper				x	
Iron		x			
Lead				x	
Mercury			x		
Selenium		x		x	
Vanadium		x			
Zinc			x	x	
Phenanthrene metabolites	x				
Benz(a)pyrene metabolites	x		x		
Liver P4501A	x	x			
Total	**8**	**5**	**11**	**7**	**0**

In addition to the above considerations that favor Four Horn Sculpin as a species for monitoring, a study in Cambridge Bay, Northwest Territories, Canada found high variability in PCBs between sites for this species separated by less than 10 km (Bright et al., 1995).

Our experience in this study is that gill nets and trawls are the best means of catching Four Horn Sculpin and other offshore fishes in the area of the platforms. Fyke nets are more appropriate for the nearshore anadramous species caught in this study.

There are some indications emerging from these data of possible anthropogenic influence in fish from the study area. Perhaps the strongest evidence is for the PAH and the markers that respond to PAH, particularly in the Four Horn Sculpin. While there were no effects of site on whole body tissue residue concentrations of total PAH or low-molecular-weight PAH in any species of fish, there were differences seen in high-molecular-weight compounds between sites for Arctic Cisco and Four Horn Sculpin. The sites where there were elevated concentrations in these two species were Stump Island and Point Brower. In Four Horn Sculpin P4501A induction in the liver cells (hepatocytes) varied significantly with site, with Point Brower and Stump Island having the highest responses. Also, phenanthrene and benz(a)pyrene equivalents in the bile varied significantly with site in Four Horn Sculpin. These are very sensitive biomarkers and the P4501A marker showed minimal levels of response over the entire area. It should also be noted that both biomarker responses can give false positive responses with regard to anthropogenic PAH:

1. P4501A can respond to other chemicals. There are potential natural inducers, i.e., peat (Kopponen et al., 1993). It is also possible that some of the pesticides that were detected in the fish tissues could have induced P4501A as not much work has been done with these compounds relative to induction of this enzyme in fish.
2. There is a background of naturally fluorescing compounds in the bile of fish that are not petroleum.

Despite these caveats, the findings with respect to PAHs are that the data are broadly consistent with accumulation of low levels of PAHs in Four Horn Sculpin, induction of the enzyme (P4501A) for metabolism of PAHs, and appearance of PAH metabolites in the bile at sites where there is known anthropogenic activity—Stump Island. We do not have an explanation for responses in Point Brower fish, but there could be influences either from the Endicott Causeway or PAHs in the Sagavanirtok River (perhaps of a natural origin). The Point Brower fish also clustered separately from other species-site combinations when only the PAHs were analyzed (Fig. 31). It should also be noted that the backward multiple regression relating tissue burdens of PAHs and PCBs to P4501A response lent a little support to the above interpretation, as the P4501A in gut mucosa of Four Horn Sculpin was significantly related to low-molecular-weight PAHs. Both these measures, LMW PAH and P4501A in gut mucosa, did not vary as function of site. One interpretation of this result of the stepwise multiple regression is that a low level of LMW PAHs occurs at some stations but not enough fish are available at the sampling sites to detect site differences. In studies of biomarkers it is not uncommon to find relationships emerging based on analysis of all individuals regardless of site that differ if the data are contrasted as site means. It should be kept in mind again here that the power of our study to resolve relationships is relatively weak for liver heptocyte P4501A and high-molecular-weight PAH due to the small number of fish of each species at our collection stations and the high variability of these measures. A clearer picture of PAH bioaccumulation and metabolism would emerge for fish by a more concentrated effort on Four Horn Sculpin.

A potentially fuller interpretation of PAHs in fish will be possible once data in the annual report for Task 2 can be compared with the fish data. There are measurements of PAHs in the sediments, amphipods and bivalves taken in Task 2 and it will be instructive to compare the variability in trace substances in invertebrates and in fish. Also, we have not attempted to apply any diagnostic ratios to determine PAH sources . Such ratios in clams, in particular, are useful, as they are likely not much altered by metabolism. It is certain however that there are both multiple natural and anthropogenic sources of PAH, in the nearshore Beaufort Sea (and that accurately distinguishing the ultimate contributions of these sources of PAH that are biologically available by tissue measurements may be impossible (Steinhauer and Boehm, 1992; Yunker and MacDonald, 1995).

Organochlorines and pesticides do not occur naturally, so what is found in the tissues is clearly anthropogenic. The only question is whether the source is distant or local, and therefore more likely to be related to industrial or military activity in oil field development area. Although distant sources are thoroughly mixed atmospherically by the time they arrive in the Arctic, there is some evidence that large rivers draining into the Beaufort Sea might result in higher local concentrations and variability within the study area that may not be attributable to local sources (Bright et al., 1995). PCBs in fish tissues in this study only varied significantly with site in Arctic Cisco. These are some of the few data on PCBs in fish from the North Slope of Alaska. In another study in the Canadian Arctic, Four Horn Sculpin were analyzed around Cambridge Bay and nearby areas, Northwest Territories that included a dump near a small town and, like Bullen Point, a DEW line site. Whole-body concentrations of PCBs (without the livers and on a wet-weight basis) ranged from about 4 to 220 ppb total PCBs. The higher values were attributed to contaminated soil near the town and the military site (Bright et al., 1995).

We have recognized two patterns of relative congener abundance in the fish from this study. The first is a mixture dominated by high-molecular-weight congeners (e.g., IUPAC congener 153). A second pattern includes a similar congener composition of high-molecular-weight compounds, but also has significant, and sometimes dominant, low-molecular-weight components (e.g. IUPAC congener 8). The low-molecular-weight congeners in such mixes have been reported previously from Beaufort Sea samples (Vallette-Silver et al., 1999). The clustering of the site-species combinations found that for PCBs, Bullen Point clustered separately, suggesting a potential local source at this site but not enough of a robust source to provide a statistically significant site difference with low numbers of fish at that site. This is also the location of an old Military installation, a Distant Early Warning (DEW) line site. There were 63 DEW line sites in the Arctic, in Greenland, Canada and Alaska and there were about 30 tons of PCBs estimated to be at these sites (AMAP, 1997).

Further, if all analytes are used, a separate cluster for Point Brower Four Horn Sculpin emerges. We have no explanation of this except that this is within the influence of the Sagavanirktok River and there are likely different ratios in the trace substances coming from this source relative to the Beaufort Sea.

For other individual classes of pesticides making up the total pesticide category there were significant differences due to site with one or more species. For Chlordanes, DDTs, Endosulfans and Endrins species which had significant variability with site had consistently high concentrations in Stump Island fish relative to those at the other sites. Point Brower fish were also high in many cases. It was also mainly the Arctic Cisco that had significant site differences for these groups of compounds, although Arctic Cod, Humpback Whitefish and Four Horn Sculpin also had a difference each for one compound or compound group. The Four Horn Sculpin had site differences for Chlordanes. Humpback Whitefish had site differences for Chlordanes and Endrins. Taken together these data suggest that there are elevated concentrations of several pesticides in the area of Stump Island and Point Brower over the general background for the area and there might be a low-level source there for pesticides. We do not know at this time what a local source might be. Pest control is one possibility that should be investigated.

Due to the natural occurrence of metals in crustal rocks and nearshore marine sediments, we face challenges in interpreting what metals may have an anthropogenic source. There were no differences due to site in the whole-body concentrations of only nickel, which will not be discussed further as there is no basis in our findings for an anthropogenic source for this metal in tissues. The remainder of the metals analyzed showed significant differences due to site in one or more species: arsenic, barium, cadmium, iron, mercury, selenium, vanadium and zinc.

The highest concentrations of arsenic occurred in Four Horn Sculpin at Stump Island and Point Brower. The source of this arsenic is uncertain, as it did not appear to be significantly elevated in sediments anywhere in the area on an iron-normalized basis (see Trefry et al, Interim Report, Task 2). There was very little variability in the concentrations of this metal in fish, so just slight changes from site to site in mean values were likely to result in finding significant differences. It is not known if there is an anthropogenic source of arsenic in the area. It should be noted that arsenic concentrations are several times greater in sediments of the sampling area than average crustal abundance and that the maximum concentration of arsenic in sediments exceeds the Effects Range Low (ERL) of Long and MacDonald (1995) (See Task 2 report—Trefry et al). It should also be noted that arsenic is generally elevated in North Slope sediments compared to other locations in Alaska (Vallette-Silver et al., 1999). A study in the eastern Beaufort Sea found concentrations of arsenic of 0.5 to 0.8 μg/g (wet-weight. basis), which compares to about 1-15 μg/g dry-weight found in this study. Using a conversion of dry to wet of about 4-5 times the values found in this present study appear to be significantly higher than those found in the previous study (West, unpublished 1985).

Also, river borne arsenic entering the Beaufort Sea on suspended particles may partition to biological compartments. Arsenic appears to be accumulated in marine organisms by trophic transfer. Most studies have found that concentrations of arsenic do not increase with increasing trophic level (e.g., Bernhard and Andrae, 1984). However, studies done in an Australian estuary suggest that biomagnification may occur (Barwick and Maher, 2003).

Analysis of the ratios of barium to iron in the fish data suggests that sediment may have played a role in trace metals detected in Four Horn Sculpin. We therefore ascribe no other particular interpretation to the site effect seen with this element in Four Horn Sculpin. Sediments do occur in the guts of bottom feeding fish. In addition, the barium sulfate used in drilling mud is not appreciably biologically available to marine organisms.

There was an effect of site on cadmium concentrations in Arctic Cisco. Point Brower fish had the highest concentrations of the four collection sites, however in the other species there was not a pronounced difference in Cd concentrations at Point Brower and other stations. Several stations had equivalent concentrations in this species. It is not known if the influence of freshwater made cadmium more biologically available at these sites.

Chromium varied significantly with site in Arctic Cisco and Four Horn Sculpin. Arctic Cisco had the highest concentrations at Stump Island. For Four Horn Sculpin, Liberty and Point Brower had the highest concentrations. We can ascribe no particular interpretation to these data.

Copper had the higher concentrations in Humpback Whitefish at Stump Island than at Point Brower. We cannot discount the possibility of an anthropogenic source of this metal at Stump Island.

Arctic Cod was the only species where the variation in iron content differed significantly between sites. Again, four of the sites had equivalent values. Iron is an essential element and is therefore physiologically regulated in fish. It seems unlikely that without a very large biologically available source that iron would appreciably accumulate in fish beyond the range that is physiologically required. However, with four of the five sites having equivalent values we ascribe no particular reason for the significant variation besides the vagaries of chance sampling and small sample sizes.

Stump Island Humpback Whitefish had higher concentrations of lead than those from Point Brower. We cannot discount the possibility of an anthropogenic source of this metal at Stump Island.

Arctic Cisco showed significant variation in mercury. Point Brower fish had greater concentrations than Stump Island fish, which in turn were higher than Liberty fish. However, the differences in means were very small. At this stage, we cannot rule out a slight local anthropogenic influence on mercury in this species. Nor can we rule out random variability in a small sample set (n=6-7 per site) as an explanation of this finding.

It should be noted that cadmium, lead and mercury are believed to be transported in the atmosphere to the Arctic, so there is a possible anthropogenic component to the concentrations in fish tissue measured here (Mac Donald et al., 2000).

For selenium, Arctic Cod from Liberty had greater concentrations than those from Northstar. Again, this is based on a very small sample size and we have no reason to attribute this to anthropogenic activities at the site.

Vanadium is another element for which Arctic Cod showed significant differences due to site. In this case, the Liberty fish had higher concentrations than the Northstar fish. Again, the potential influence of sediments in the gut, as well as a small sample size, may be a factor as there are no known sources of vanadium at Liberty.

For zinc, the Arctic Cisco and the Humpback Whitefish had variation due to site. In this case Stump Island fish had consistently higher concentrations.

Among all of the analytes assessed by Task 8, PCBs appear to be of greatest concern to human health based on measured whole-body concentrations. When whole body tissue residues are compared to EPA's screening values for subsistence consumption of (fish) flesh, it appears that flesh concentrations, had we measured them, might approach EPAs screening values, which at 3 ppb total PCBs, are conservative in comparison with FDA screening values of 2 ppm. The data produced by Task 8 investigations cannot be compared to EPA and FDA screening values, as Task 8 data are from whole-body concentrations and EPA and FDA values are based on concentrations in muscle tissue. PCBs are stored in lipid-rich tissues, and are typically in much lower concentrations in flesh than in whole body. Additionally, EPA subsistence screening values are based on a detailed risk assessment scenario that applies risk based on specified levels of consumption. The data produced in Task 8 is based only on whole body fish tissue concentrations. No investigation of consumption levels was included. National Status and Trends Benthic Surveillance Project fish data was investigated to determine if useful comparisons could be made between the fish from the North Slope and multiple locations from around the United States. However, after careful consideration of the NS&T data we decided not to use it, as all of the PCB data produced by NS&T is from fish livers rather than whole-body concentrations. Fish liver concentrations are expected to be much higher (perhaps an order of magnitude or more) than whole-body concentrations.

Ecological effects of PCBs in fish are a prominent concern in addition to the obvious concerns to subsistence consumers. The vast majority of studies have human health as their main priority and therefore investigate PCBs only in the edible portions (flesh) of the investigated organisms. This is unfortunate (from our perspective) from several standpoints. Chief among them is that PCBs accumulate most heavily in the lipid rich portions (organs), making the data non-comparable with studies such as this one, the objective of which was determination of whole body levels, thus allowing an investigation of the environment with human health issues taking a lesser role. From a purely ecological perspective, one recent study (Meador et. al., 2002) emerges that looked at whole body tissue residues of PCBs in salmonids. The authors investigated all of the effects studies conducted to date and determined (using the admitted conservative 10^{th} percentile approach recommended by the EPA) that whole body lipid normalized wet-weight. concentrations of total PCBs exceeding 2.4 ppm cause harm (as defined in the Endangered Species Act) to juvenile salmonids. While the Meador et. al., study did not include Whitefish or Cisco, they are closely related to the Salmon, Trout and Char that were investigated. We clearly understand that significant physiological differences between groups of fishes make a comparison of potential harm somewhat tenuous, however given the lack of available data, we think a comparison is worthwhile in this case. The mean tissue

concentrations of PCBs for all five of the (Task 8) investigated species were below 2.4 ppm. The Four Horn Sculpin at Bullen Point had a mean value of 2.3 ppm, and a single individual had a concentration of 462 ppm. Sculpins at Bullen Point clearly had the highest PCB concentrations of any of the site-species combinations. The concentrations found there are of some concern for ecological effects on fish. (Hoekstra et. al., (2003) also found concentrations well bellow 2.4 ppm in fish collected by subsistence fishers from nearby Elson lagoon (Barrow, AK). Additionally, a study by Atuma et. al., (1998) documented whole body lipid normalized wet-weight. concentrations of 1.3-3.1 ppm PCBs in Atlantic Salmon from the Baltic Sea. Further investigation of contaminants and their potential effects on fish (e.g., in Four Horn Sculpin) is warranted based on findings presented here.

5.0 References

Arctic Monitoring and Assessment Program. 1997. Arctic Pollution Issues: A state of the Arctic environmental report. AMAP, Oslo, Norway.

Arctic Monitoring and Assessment Program. 1999. Heavy Metals in the Arctic. Proceedings of the International Workshop, November 1999, Anchorage, Alaska. September 7-10 1999.

Arthur D. Little, Inc. 2001. 2001 Field Sampling and Logistics Plan for the Minerals Management Service ANIMIDA Field Survey. Prepared for Minerals Management Service. July.

Atuma, S. S., Aune, M., Bergh, A., Wicklund-Glynn, A., Darnerud, P. O., Larson, L., Olsson, M., and Sanstrum, O. 1998. Polychlorinated biphenyls in salmon (*Salmo salar)* from the Swedish east coast. *Organhalogen Compounds,* v. 39, pp. 153-156.

Barber, W.E., Smith, R.L., Vallarino, M. 1997. Demersal fish assemblages of the northeastern Chukchi Sea, Alaska. *Fisheries Bulletin,* v. 95, pp. 195-209.

Barwick, M. and Maher, W. 2003. Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Mar. Environ. Res.,* v. 56, pp. 471-502.

Bernhard, M. and Andreae, M.O. 1984. Transport of trace metals in marine food chains. *Changing Metal Cycles and Human Health.* Nriagu, J.O. (ed.) 143-167. Dahlen Konferenzen, Berlin.

Bright, D.A., Dashenko, W.T., Grundy, S.L. and Reimer, K.J. 1995. Effects of local and distant contaminant sources: polychlorinated biphenyls and other organochlorines in bottom-dwelling animals from an Arctic estuary. *Sci. Total Environ*., v. 1995, pp. 265-283.

Brown, J.S., Boehm, P.D., Trefrey, J.H., Cook, L.L., Smith, W.K. 2002. ANIMIDA Task 2: Hydrocarbon and Metal Characterization of Sediments, Bivalves and Amphipods in the ANIMIDA Study Area. Interim Report to U.S. Department of the Interior, Minerals Management Service, Anchorage, Alaska.

Craig, P.C. 1984. Fish Use of Coastal Waters of the Alaskan Beaufort Sea: A Review. *Transactions of the American Fisheries Society,* v. 113, pp. 265-282.

Craig, P.C. 1989. An Introduction to Anadromous Fishes in the Alaskan Arctic. *Biol. Pap. Univ. Alaska.,* v. 24, pp. 27-54.

Elskus, A. and Stegeman, J.J. 1989. Induced cytochrome P4501A in *Fundulus heteroclitus* associated with environmental contamination by polychlorinated biphenyls and polynuclear aromatic hydrocarbons. *Mar. Environ. Res.*, v. 27, pp. 31-50.

Fechhelm, R.G. 1999. The Effect of New Breaching in a Prudhoe Bay Causeway on the Coastal Distribution of Humpback Whitefish. *Arctic,* v. 52, pp. 386-394.

Fechhelm, R.G. and Griffiths, W.B. 1990. Effects of Wind on the Recruitment of Canadian Arctic Cisco (*Coregonus autumnalis)* into the Central Alaskan Beaufort Sea. *Can. J. Fish. Aquat. Sci.,* v. 47, pp. 2164-2170.

Fechhelm, R.G., Bryan, J.D., Griffiths, W.B., Wilson, W.J., Gallaway, B.J. 1994. Effect of Coastal winds on the Summer Dispersal of Young Least Cisco *(Coregonus sardinella)* from the Colville River to Prudhoe Bay, Alaska: A Simulation Model. *Can. J. Fish. Aquat. Sci.,* v. 51, pp. 890-899.

Fechhelm, R.G., Griffiths, W.B., Matin, L.R., Gallaway, B.J. 1996. Intra- and Interannual Variation in the Relative Condition and Proximate Body Composition of Arctic Ciscoes from the Prudhoe Bay Region of Alaska. *Transactions of the American Fisheries Society*, v. 125, pp. 600-612.

Fechhelm, R.G., Griffiths, W.B., Wilson, W.J., Gallaway, B.J., Bryan, J.D. 1995. Intra- and Interseasonal Changes in the Relative Condition and Proximate Body Composition of Broad Whitefish from the Prudhoe Bay Region of Alaska. *Transactions of the American Fisheries Society,* v. 124, pp. 508-519.

Fechhelm, R.G., Griffiths, W.B., Bryan, J.D., Gallaway, B.J., Wilson, W.J. 1995. Application of an In Situ Growth Model: Inferred Instance of Interspecific Competition between Anadroumous Fishes of Prudhoe Bay, Alaska. *Transactions of the Amercan Fisheries Society,* v. 124, pp. 55-69.

Fechhelm, R.G., Dillinger, R.E. Jr., Gallaway, B.J. 1992. Modeling of In Situ Temperature and Growth Relationships for Yearling Broad Whitefish in Prudhoe Bay, Alaska. *Transactions of the American Fisheries Society,* v. 121, pp. 1-12.

Fechhelm, R.G., Martin, L.R., Gallaway, B.J., Wilson, W.J., Griffiths, W.B. 2001. Estimating the Hydrographic Effects of Prudhow Bay Causeway Breaches Using Before-After Control Impact (BACI) Analysis. *Arctic,* v. 54, pp. 162-173.

Fechhelm, R.G., Martin, L.R., Gallaway, B. J., Wilson, W.J., Griffiths, W.B. 1999. Prudhoe Bay Causeways and the Summer Coastal Movements of Arctic Cisco and Least Cisco. *Arctic,* v. 52, pp. 139-151.

Florio, S.A. 2003. personal communication. S.A. Florio, P.E. Ecology and Environment 840 K Street, Anchorage, AK 99501 (907) 257-5000. sflorio@ene.com.

Gerrodette, T. 1987. A power analysis for detecting trends. *Ecology*, v. 68, pp. 1364-1372.

Goldberg, S.R., Yasutake, W.T., West, R.L. 1987. Summer spawning in the fourhorn sculpin, *Myoxocephalus quadricornis*, from Alaska. *Can. Field-Nat*., v. 101, p. 457

Gonzalez, F.J. 1989. The molecular biology of cytochrome P450s. *Pharmacol. Rev*., v. 40, pp. 243-288.

Graham, M. and Hop, H. 1995. Aspects of reproduction and larval biology of Arctic cod (*Boreogadus saida)*. *Arctic*, v. 48, pp. 130-135.

Griffiths, W.B., Fechhelm, R.G., Gallaway, B.J., Martin, L.R., Wilson, W.J. 1998. Abundance of Selected Fish Species in Relation to Temperature and Salinity Patterns in the Sagavanirktok Delta, Alaska, Following Construction of the Endicott Causeway. *Arctic,* v. 51, pp. 94-104.

Griffiths, W.B., Gallaway, B.J., Gazey, W.J., Dillinger, R.E. Jr. 1992. Growth and Condition of Arctic Cisco and Broad Whitefish as Indicators of Causeway-Induced Effects in the Prudhoe Bay Region, Alaska. *Transactions of the American Fisheries Society,* v. 121, pp. 557-577.

Hall, L.W. Jr; Anderson, R.D. 1995. The influence of salinity on the toxicity of various classes of chemicals to aquatic biota. *Crit. Rev. Toxicol*., v. 25, pp. 281-346.

Herbert, C. E. and Keenleyside, K. A. (1995). To normalize or not to normalize? Fat is the question. *Environmental Toxicology and Chemistry,* v. 14, pp. 801-807.

Hoekstra, P. F., O'hara, T.M., Fisk, A.T., Borga, K., Solomon, K.R., Muir, D.C.G. 2003. Trophic Transfer of Persisitent Organochlorine Contaminants (OCs) Within an Arctic Marine Food Web from the Southern Beaufort-Chukchi Seas. *Environmental Pollution*, v. 124, pp. 509-522.

Kline, T.C., Wilson, J.J, Goering, J.J. 1999. Natural isotope indicators of fish migration at Prudhoe Bay Alaska. *Can. J. Fish*., v. 55, pp. 1494-1502.

Kloepper-Sams, P.J., Stegeman, J.J. 1989. The temporal relationships between P450E protein content, catalytic activity, and mRNA levels in the teleost *Fundulus*

heteroclitus following treatment with beta-naphthoflavone. *Arch. Biochem. Biophys*., v. 268, pp. 525-235.

Kopponen, P., Tarhanen, J., Ruuskanen, J., Torronen, R., Karenlampi, S. 1993. Peat induces cytochrome P450IA1 in Hepa-1 cell line. Comparison with fly ashes from combustion of peat, coal, heavy fuel oil and hazardous waste. *Chemosphere,* v. 26, pp. 1499-1506.

Krahn, M.E. and Malins, D.C. 1982. Gas chromatographic-mass spectrometric determination of aromatic hydrocarbon metabolites from livers of fish exposed to fuel oil. *J. Chromatog.,* v. 248, pp. 99-107.

Krahn, M.E., Meyers, M.S., Burrows, D.G., Malins, D.C. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. *Xenobiotica.,* v. 14, pp. 633-664.

Long, E.R., MacDonald, D.D, Smith, S.L., Calder, F.D. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management,* v. 19, pp. 81-97.

McCain, B.B., Hodgkins, H.O., Gronlund, W.D., Hawkes, J.W., Brown, D.W., Meyers, M.S. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (*Parophrys vetulus*), and pathological consequences. *J. Fish. Res. Board Can.,* v. 35, pp. 657-664.

MacDonald, R.W., Barrie, L.A., Bidleman, T.F., Diamond, M.L., Gregor, D.J., Semkin, R.G., Strachan, W.M.J., Li, Y.F., Wania, F., Alaee, M., Alexeeva, L.B., Backus, S.M., Bailey, R., Bewers, J.M. 2000. Contaminants in the Canadian Arctic: 5 years of progress in understanding sources, occurrence and pathways. *Sci. Total Environ*., v. 2-3, pp. 93-234.

Meador, J. P., Collier, T.K., Stein, J.E. 2002. Use of tissue and sediment based threshold concentrations of polychlorinated biphenyls (PCBs) to protect juvenile salmonids listed under the US Endangered Species Act. *Aquatic Conserv: Mar. Freshw. Ecosyst.,* v. 12, p. 493-516.

Prentki, R. 2001. personal communication. R. Prentki. U.S. Minerals Management Service, Alaska Region. 949 E. 36th Ave. Anchorage, AK. 99508-4363. (907) 271-6621. richard.prenkti@mms.gov.

Schmidt, D.R., Griffiths, W.B., Martin, L.R. 1989. Overwintering Biology of Anadromous Fish in the Sagavanirktok River Delta, Alaska. *Biol. Pap. Univ. Alaska.* No. 24, pp. 55-74.

Smolowitz, R.M., Hahn, M.E., Stegeman, J.J. 1991. Immunochemical localization of cytochrome P450IA1 induced by by 3,3',4,4-tetrachlorobiphenyl and by 2,3,7,8-tetrachlorodibenzofuran in liver and extrahepatic tissues of *Stenotomus chrysops* (scup). *Drug. Metab. Dispos.,* v. 19, pp. 113-123.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*, third edition. W. H. Freeman and Company, New York.

Spies, R. B., Felton, J.S, Dillard, J.L. 1982. Hepatic mixed function oxidases in California flatfish are increased in contaminated environments and by oil and PCB ingestion. *Mar. Biol*., v. 70, pp. 117-127.

State of Alaska. 2000. DHSS DPS Section of Epidemiology. Health Consultation Interim Report Former Umiat Air Force Station, Umiat Alaska. Anchorage , AK

Steinhauer, S.S. and Boehm, P.D. 1992. The composition and distribution of saturated and aromatic hydrocarbons in nearshore sediments, River sediments and Coastal Peat of the Alaskan Beaufort Sea: Implications for detecting anthropogenic hydrocarbon inputs. *Marine Environ. Research.,* v. 33, pp. 223-253.

U.S. EPA. 1993. Test Methods for Evaluating Solid Waste, SW-846, Third Edition. Office of Solid Waste, Washington, D.C., August.

Valette-Silver, N., M.J. Hameedi, D.W. Efurd and A. Robertson. 1999. Status of the contamination in sediments and biota from the Western Beaufort Sea (Alaska). *Mar. Poll. Bull.,* v. 38, pp. 702-722.

Yunker, M. B. and MacDonald, R.W. 1995. Composition and origins of polycyclic aromatic hydrocarbons in the McKenzie River and on the Beaufort Sea Shelf. *Arctic,* v. 48, pp. 118-129.

West, R. L. 1985. Baseline histopathological and contaminant studies of four Arctic fish species in Beaufort Lagoon, Arctic National Wildlife Refuge, Alaska. Unpublished report, US Fish and Wildlife Service, Fairbanks, AK.

SAS Institute. (2000). JMP Statistical Discovery Software, Cary, NC 27513.

Sokal, R. R. & ROHLF, F. J. (1995). *Biometry*, third edition. W. H. Freeman and Company, New York.

6.0 Appendices